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THE EFFECT OF CONCENTRATES OF HIGH OR LOW STARCH EQUIVALENT, BOTH FED AT TWO LEVELS, ON THE MILK PRODUCTION OF DAIRY COWS

BY M. E. CASTLE, D. S. MACLUSKY,* J. MORRISON† AND J. N. WATSON

The Hannah Dairy Research Institute, Kirkhill, Ayr

Recent winter-feeding experiments with the Hannah Institute herd have suggested that with a basal ration of good quality grass feeds certain economies could be made in the amount and quality of concentrates fed in mid-lactation without loss of milk production. The factors so far investigated have been the protein content of the concentrate (1), the relative effects of two protein contents at two levels of feeding (2), the starch equivalent of the concentrate (1), and lastly the effect of feeding a medium quality concentrate at four different levels (3). In the experiment reported here the effects of feeding concentrates of high or low starch equivalent, both at two levels, on milk yield and quality in mid-lactation were studied.

EXPERIMENTAL

Experimental design

Eight Ayrshire cows, which had calved from one to three months before the experiment began, were used. Five of them were in their second lactation and the others were in their fourth or fifth lactation. They were housed throughout the experiment, apart from a period of 1–3 hr. each day when they were allowed exercise in a bare paddock.

The cows were divided into two groups of four so that the members in each group corresponded as far as possible in stage of lactation and in milk yield during the month preceding the experiment.

The four cows in each group were allotted at random to each treatment in a balanced complementary 4 × 4 Latin square (4) as follows, A, B, C and D denoting the four treatments:

	Periods			
Cows	1	2	3	4
1	A	B	C	D
2	B	D	A	C
3	C	A	D	B
4	D	C	B	A

Each period lasted 3 weeks, and the changeover from one feeding treatment to another was made gradually over a period of 4 days. The results from the last 2 weeks only of each period were used in assessing the influence of the different treatments on milk yield and composition, live weight and food consumption.

* Present address: King's College, Newcastle. † Colonial Office Student.

Feeding

The four feeding treatments as planned were:

A 4·5 lb./gal. of high starch equivalent concentrate (79 S.E.).
B 4·5 lb./gal. of low starch equivalent concentrate (63 S.E.).
C 2·5 lb./gal. of high starch equivalent concentrate (79 S.E.).
D 2·5 lb./gal. of low starch equivalent concentrate (63 S.E.).

In the experiments it was found that the average amounts of concentrates consumed per gallon were very slightly higher than the amounts stipulated, being 4·6 and 2·6 lb. respectively. Details of the two concentrates are given in Table 1. The concentrates were prepared from commonly available constituents in such a way that while they were similar in dry matter and digestible crude protein contents, the difference between them in calculated starch equivalent (63 and 79) was about as great as is likely to be found in concentrates available to the farmer. The starch equivalent and protein contents were calculated from the values of the individual ingredients given in the tables by Woodman (5).

Table 1. *Ingredients and composition of the concentrate mixtures*

	High starch equivalent mixture (lb.)	Low starch equivalent mixture (lb.)
Flaked maize	68	21
Decorticated groundnut cake	8	21
Locust beans	15	—
White fish meal	9	—
Oats (crushed)	—	32
Bran	—	26
Dry-matter content (%)	85·0	85·7
Calculated starch equivalent	79·0	63·0
Calculated digestible protein content (%)	15·3	14·6

The weight of concentrates fed to each cow daily at either 2·6 or 4·6 lb./gal. of milk was kept constant during each period, and was adjusted at the beginning of each period according to the average decline in milk yield of the cows on the higher level of feeding during the preceding period (6). This was done in order to avoid the possibility of sharp declines in yield as the experiment progressed.

The basal ration fed to each cow daily was as follows: 7–10 lb. hay, 4 lb. artificially dried grass, 30 lb. grass silage and 20 lb. fodder beet.

The dry matter, crude protein and crude fibre contents of these feeds were determined and are shown in Table 2. The starch equivalent and digestible crude protein values assigned to each food for the purpose of calculating the apparent intakes of starch equi-

Table 2. *Composition of the foods in the basal ration* (%)

		In the dry-matter		In the food	
	Dry-matter	Crude protein	Crude fibre	Starch equivalent	Digestible crude protein
Hay	85·4	8·2	32·7	24·0	4·0
Dried grass	90·4	15·1	27·0	58·0	9·5
Grass silage	20·9	11·4	29·0	10·5	1·6
Fodder-beet (Yellow-Daeno)	20·0	5·5	5·0	10·0	0·4

valent and digestible crude protein are also given. The sources of these values were as follows: for hay, Woodman (5), for dried grass, Watson & Horton (7), for grass silage, Holmes (8), and for fodder-beet, Bailey, Castle & Foot (9). The hay, although well made, was mature and stemmy, with a crude fibre content in the dry matter of 33%, consequently 4 lb. of dried grass were fed with it so as to simulate a ration of high quality hay. The average starch equivalent of the combined ration of hay and dried grass consumed was calculated to be 35. The silage consisted almost entirely of grass cut at the early flowering stage and contained virtually no clover. No preservative was used in making it, and the fermentation was satisfactory.

The cows were fed individually, and all food offered and refused was weighed daily. The silage was fed immediately after the morning milking, the fodder-beet at noon and the hay and dried grass after the afternoon milking. The weights of silage, dried grass and fodder-beet offered were the same for all the cows, but the amount of hay fed was adjusted according to appetite by offering it in quantities that always ensured some measurable residue.

The hay, dried grass, fodder-beet and concentrates were sampled for the determination of dry matter content once, and the silage three times, each week. Their crude fibre and crude protein contents were determined from the weekly sample for each food except silage, for which a bulked sample was made each week from the 3 days' samples.

Milk yield and quality

The cows were milked twice a day and their yields recorded at each milking to the nearest $\frac{1}{4}$ lb. Milk samples for the determination of fat and solids-not-fat were taken at one evening milking and at the following morning milking once per week. The fat content of the milk was determined by the Gerber method, and the solids content gravimetrically. To detect and control any mastitis that might occur, samples of milk were regularly examined by Dr P. S. Blackburn of this Institute, using the differential cell-count method of Blackburn, Laing & Malcolm (10).

In period 2, one cow in group 1 had a severe digestive disturbance, and a cow in group 2 accidentally consumed a large weight of concentrates. As a result there were large but temporary drops in milk yield, and 'missing values' were calculated for the milk yields of these two cows in this period.

Live weights

The cows were weighed on three consecutive afternoons each week immediately before the afternoon milking.

RESULTS

Feed consumption

Dry matter. The average amounts of each of the foods consumed by the cows on each treatment are shown in Table 3. The weights of fodder-beet and dried grass consumed were identical on each treatment and there were only small and statistically non-significant differences in the amounts of hay and silage consumed. The difference in total dry-matter intake of $6{\cdot}8 \pm 0{\cdot}5$ lb. between treatments A and B on the one hand and C and D on the other, was attributable almost entirely, therefore, to the difference in the amount of concentrate dry matter consumed. There was no difference between the two qualities of concentrate in their effect on dry-matter intake. The average daily dry-matter intake

from all feeds was equivalent to 2·6% of the cows' live weights. At the higher level of concentrate feeding this value was 2·9% and at the lower level 2·4%.

Table 3. *The average weight of each food, the dry matter, the starch equivalent and the digestible crude protein consumed on the four feeding treatments*

(lb./cow/day)

Treatment ...	A	B	C	D
Concentrates	15·0	15·2	7·8	7·8
Basal feeds				
Fodder-beet	20·0	20·0	20·0	20·0
Silage	26·5	26·7	28·0	27·6
Hay	7·0	7·1	6·9	7·1
Dried grass	4·0	4·0	4·0	4·0
Dry matter				
Basal feeds	19·3	19·1	19·4	19·3
Total*	32·4	32·4	25·4	25·7
Starch equivalent†	21	19	15	14
As % of Woodman's standards (5)	139	122	106	97
Digestible crude protein	3·5	3·5	2·4	2·4

* Standard error of difference between two means = ±0·5.
† Standard error of difference between two means = ±0·3.

Digestible crude protein. Since both the concentrate mixtures had similar digestible crude protein contents, there was no change in digestible crude protein intake when a change was made from one concentrate mixture to another at the same level of feeding. However, the greater amounts of concentrate consumed on treatments A and B, compared with those on C and D, resulted in an increase in digestible crude protein consumption from an average of 2·4 to an average of 3·5 lb./cow/day when the greater amounts were fed.

Starch equivalent. The intake of starch equivalent was calculated from the dry-matter intake of each feed, using the starch equivalent values given in Tables 1 and 2. The differences between the two qualities of concentrate and also between the two rates of feeding were both statistically significant ($P < 0{\cdot}01$). These amounts increased from a minimum of 14 lb./cow/day on treatment D to a maximum of 21 lb. on treatment A. An increase in the amount of concentrate fed resulted in a greater increase in starch equivalent intake than did an increase in the starch equivalent content of the concentrate.

Level of feeding. The amounts of starch equivalent consumed expressed as a percentage of Woodman's standards for the cow's requirements (5) are shown in Table 3. The level of feeding was 97% of Woodman's standards on treatment D (the low starch equivalent concentrate fed at the low rate) and rose to a maximum of 139% on treatment A.

Milk yield

High cell counts and some organisms were found in the milk from a number of quarters which were treated subsequently with antibiotics, but no clinical symptoms of mastitis or other abnormality were observed. In view of this it is not considered that any depression in milk yield or change in milk composition associated with the high cell counts would be sufficient to influence significantly the results obtained and the conclusions arrived at in the present experiment.

The mean daily milk yields per cow on each treatment are given in Table 4. When concentrates were fed at the higher rate in treatments A and B the average daily milk

yield was 34·7 lb., being 3·8 lb. more than the yield at the lower rate in treatments C and D. This difference was statistically significant at the 1% level. There was a statistically non-significant difference in yield of 1·0 lb. milk/cow/day between treatments A and B in which the high and low S.E. concentrates were fed at the high level. Similarly, when they were fed at the low level there was a non-significant difference of 0·6 lb. No significant residual effects of the treatments from one period to another were found when the data were analysed by the method of Williams (4). The coefficient of variation, 4·4%, was somewhat higher than in previous experiments of this type on the same herd, but this was probably due to missing values for two cows in the second period.

Table 4. *The mean daily milk yield, the fat and solids-not-fat content of the milk and the live weights of the cows*

Type of concentrates	Rate of feeding (lb./gal.)	Mean milk yield (lb./cow/day)	Mean fat (%)	Mean solids-not-fat (%)	Mean live weight (lb.)
A. High starch equivalent	4·6	34·2	3·83	8·77	1112
B. Low starch equivalent	4·6	35·2	3·88	8·67	1117
C. High starch equivalent	2·6	30·6	3·84	8·61	1080
D. Low starch equivalent	2·6	31·2	3·79	8·53	1083
Standard error of difference between two means	—	±0·7	±0·13	±0·05	±7

Milk quality

The mean fat and solids-not-fat contents of the milk on each treatment are shown in Table 4.

When the rate of concentrate feeding increased from 2·6 to 4·6 lb./gal. of milk, there was an increase in the solids-not-fat content of the milk from a mean value of 8·57 to one of 8·72%. This increase of 0·15% was statistically significant ($P < 0·01$). The smaller differences of 0·10 and 0·08 between the mean solids-not-fat percentage of the milk produced with each of the two qualities of concentrate were not statistically significant. The coefficient of variation was 0·8%.

There were no significant differences between the treatments in the fat content of the milk.

Live weights

The average live weights are shown for each treatment in Table 4. There was virtually no difference in live weight attributable to the starch equivalent content of the concentrates, i.e. treatment A did not give a higher mean live weight than treatment B, and treatment C did not give a higher value than treatment D, but there was an average difference in live weight of 33 lb./cow between the high and low rates of concentrate feeding. This difference was statistically highly significant ($P < 0·01$).

DISCUSSION

The results of this trial showed that on a basal ration of hay, silage, roots and a little dried grass, an increase from 2·6 to 4·6 lb. in the amount of concentrates fed per gallon of milk gave an average increase of 3·8 lb. in the daily milk yield per cow, and an increase of 0·15 in the solids-not-fat percentage in the milk. An increase in the starch equivalent content of the concentrates from 63 to 79 gave no increase in milk yield at either the high

or low rate of feeding, and gave only a small and non-significant increase in mean solids-not-fat content. This result can be better understood when the relative effects of quality and quantity of concentrate on the daily starch equivalent and digestible crude protein consumption are considered. It can be calculated from the values for total starch equivalent intake summarized in Table 3 that changing from the low to the high starch equivalent concentrate increased the daily starch equivalent consumption by 8·6% at the 2·6 lb. level of feeding (D to C) and by 12·3% at the 4·6 lb. level of feeding (B to A), whereas changing the rate of feeding from 2·6 to 4·6 lb./gal. of milk increased the starch equivalent consumption by 33·8% on the low starch equivalent concentrate (D to B) and by 38·4% on the high starch equivalent concentrate (C to A). In addition, as both concentrates had similar protein contents, and the weights of food consumed on the basal ration on all four treatments were almost identical, there was no change in digestible crude protein intake when a change was made from one concentrate to another (A to B, and C to D) at the same level of feeding. When, however, the level of concentrate feeding was increased from 2·6 to 4·6 lb./gal. there was an increase in digestible crude protein consumption from 2·4 lb./day on both treatments C and D to 3·5 lb./day on treatments A and B.

Thus at both the low and the high level of concentrate feeding the total effect of the increase in the starch equivalent content of the concentrate (D to C and B to A) was relatively small and had a non-significant effect on both the milk yields and the solids-not-fat content of the milk. When the level of concentrate feeding was increased, and both the starch equivalent and protein intake increased there were substantial increases in both the milk yield and the solids-not-fat content of the milk. The lowest level of protein intake, that on treatments C and D, was equal to the standards of Woodman (5), but on treatments A and B it was about 140% of these standards. In previous work on the same herd (1, 2) the level of protein intake within the limits of the experiments had only a slight effect on milk yield and composition, but in the present work the higher yields obtained on treatments A and B may have been due in part to the higher protein intake as well as to the higher intake of starch equivalent.

Since the difference in starch equivalent content between the two concentrates was about as great as is likely to be found with commercially available feeds, these results suggest that in practice an increase in milk yield and solids-not-fat content may be more easily obtained by increasing the amount of concentrate fed than by increasing the starch equivalent of the concentrate. With very high yielding cows, however, where the intake of nutrients must necessarily be much greater the starch equivalent content of the concentrate might well be more important than it was in this experiment. The effect on the milk yield and solids-not-fat content of increasing the rate at which concentrates were fed was possibly magnified by the fact that when concentrate consumption increased by 7·3 lb./cow daily there was little or no corresponding reduction in the amount of the basal ration consumed. In previous experiments with this herd (2, 3) and elsewhere (11), an increase in the rate of concentrate feeding caused a decline in the amount of basal feed consumed of from 40 to 60 lb./100 lb. of additional concentrate.

When feeding costs and returns are considered, it is obvious that since increasing the starch equivalent content of the concentrate failed to increase the milk yield, there could be no economic justification for feeding it. It is even doubtful whether the increase in yield resulting from the increased rate of feeding could be economically justified since the

feeding of each additional 1 lb. of starch equivalent gave increases of only 0·7 lb. milk and 0·03% in the percentage of solids-not-fat in the milk. This is confirmed when the costs of feeding and the value of the milk produced on each treatment are compared at current prices and costs. The greatest profit margin, i.e. the value of the milk less the cost of the food, was found with treatment D (low starch equivalent concentrate fed at the low rate) and the smallest with treatment A (high starch equivalent concentrate fed at the high rate). These profit margins, however, are not necessarily those that might have been obtained if the same treatments had been applied throughout the lactation when the effects of the treatments on the body condition of the cows might become important. In this connexion it should be noted that there was a difference between the mean live weights on the high and low levels of concentrate feeding. It was not possible to determine any consistent differences between each of the treatments in their effects on the daily rate of change of live weight except that all but the highest levels of feeding showed small negative values. This suggests that the true starch equivalents of the feeds as fed were, in fact, slightly lower than the values assumed in Table 2. Moreover, with the cows used in this experiment the mean solids-not-fat content of the milk at the lowest level of feeding (8·53%) barely exceeded the presumptive legal standard of 8·50%, and although this treatment gave the highest profit margin, there was a constant danger with these particular cows of the solids-not-fat content falling below 8·50%.

Since the loss in live weight on the lower levels of feeding, although slight, might ultimately have had a detrimental effect on milk production or have involved subsequent expense in making good the loss, and since increasing the starch equivalent of the concentrates made little difference to milk yield, milk quality or live weight, it is concluded that of the four treatments the optimal one, under the conditions of this experiment and with the qualities of roughage used here, was 4·6 lb./gal. of low starch equivalent concentrate (treatment B), but it is probable that in actual practice over a complete lactation a somewhat smaller allowance would be adequate.

SUMMARY

1. In a winter feeding trial, using eight Ayrshire cows, two concentrates with digestible crude protein contents of 15% and starch equivalents of 63 and 79 were each fed at 2·6 and 4·6 lb./gal. of milk.

2. The basal ration was hay, silage, dried grass and fodder-beet.

3. Milk yield was significantly increased from 30·9 to 34·7 lb./day when the amount of concentrate fed increased from 2·6 to 4·6 lb./gal., but the average milk yields when the concentrates low in starch equivalent were fed did not differ significantly from those obtained when the concentrates high in starch equivalent were fed at the same levels.

4. The fat content of the milk was similar on all four rations with an average value of 3·84%, whereas the solids-not-fat varied from 8·53% on the low starch concentrate fed at the lower rate to 8·77% on the high starch concentrate fed at the higher rate.

5. The cows which were fed at the rate of 4·6 lb./gal. were significantly heavier by an average of 33 lb./cow than the ones fed at the rate of 2·6 lb./gal. The two different qualities of concentrate did not cause differences in live weight.

6. The weight of dry matter in the basal ration consumed per day was similar on all four treatments.

7. A comparison of feeding costs and the value of the milk showed that the largest margin was obtained with the low starch equivalent concentrate fed at the rate of 2·6 lb./gal.

8. It was concluded that because of the loss in live weight on the lowest level of feeding, and because the quality of the concentrate gave no improvement in milk yield or live-weight and little improvement in milk composition, the optimal level of feeding in this experiment was 4·6 lb./gal. of the low starch equivalent concentrate.

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AUTOGENOUS TOXOID VACCINE IN THE PROPHYLAXIS OF STAPHYLOCOCCAL MASTITIS IN CATTLE

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There are few references to the use of staphylococcal vaccines and toxoids in the prophylaxis of mastitis in cattle. Kearney & Moloney (1) carried out a 16-month survey in a herd of 80 cows following the use of an autogenous staphylococcal toxoid and streptococcal 'anaculture'. Thirty-eight of the cows were clinically affected with mastitis before vaccinations started and 22 of them were carrying staphylococci, streptococci or both. Three courses of vaccine were given over a period of nine months and it is claimed that the majority of the infections were eliminated and that any new clinical cases were less severe than previously.

Collingson (2), Gould (3) and Wilson (4) have reported on the therapeutic use of staphylococcal toxoid in bovine mastitis and claim varying degrees of success. Their results are based mainly on clinical improvement.

Little & Plastridge (5) summarized the position in 1946 and said that previous work suggested that staphylococcal toxoids were unlikely to be of value in the prevention of 'ordinary' staphylococcal mastitis.

Recent literature is more encouraging and offers a more fundamental approach to the problem in cattle, though emphasis is still placed on the therapeutic value of toxoids rather than on their prophylactic value. Ramon, Richou and their associates (6,7,8) have studied the use of toxoid with and without the incorporation of bacterial bodies and potassium alum, and found the latter two substances to have an adjuvant effect. A higher and more persistent antibody titre was obtained than by the use of toxoids alone. Their report in 1953 suggests that alpha and beta toxoids should both be used in the treatment of cases, and that killed staphylococci or coliform bacteria should be added to the toxoid. Their work on staphylococcal antibody production in cattle is very important and gives a sound basis for the use of vaccines in the prophylaxis of mastitis.

In the present paper the system of injections used is based on the observation of Ramon *et al.* (6) that a good antitoxin response follows three to four injections of toxoid at 5-day intervals, and that a single injection of toxoid will boost the waning titre several months later.

Spencer, Stewart & Lasmanis (9) have issued a preliminary report on the application of toxoids and vaccines in cattle, assessing their value on the protection given against a challenge infection in the udder. A dried detoxified culture suspended in aluminium hydroxide and given intramuscularly appears to have been most suitable and to have given good protection in six cattle compared with the reaction following challenge in two controls. Five of the six cattle showed a good early antitoxin response, though this waned considerably by the 14th week. Sixteen quarters of four vaccinated cows were challenged by intramammary inoculation of broth culture either 21 days or 4 months after vaccination,

but the reactions were not marked in any instance. Nine of the infections persisted, yet only one of these was in a cow showing negligible antitoxin response. The opinion of the authors is that 'whole cultures inactivated and detoxified seem to offer more promise for immunization of cattle than do purified toxoids'.

EXPERIMENTAL

The present studies commenced in a herd in November 1955 when it was decided to vaccinate a group of first and second calving cows with an autogenous vaccine/toxoid prepared from coagulase positive strains of *Staphylococcus aureus* isolated from udders in the herd.

The herd had a high incidence of mastitis in 1953, the predominating organism being a staphylococcus. The mastitis was never acute, and arose in subacute form usually a few weeks after calving, assuming then a chronic intermittent character in the affected cow. Quarters clinically affected were prone to further attacks and liable to retain the staphylococcal infection. In some clinical cases in the herd *Streptococcus agalactiae* was present and occasionally this organism was found to precede or replace staphylococcal infections in quarters under study. Clinical mastitis was not so high in 1954 or 1955, but there was still a noticeable incidence of new staphylococcal infections.

The staphylococci were predominantly of one bacteriophage type (52 B, 29, 42 E, 42 F, 1, 8, 10) and resistant both clinically and *in vitro* to penicillin. Only one cow (746) constantly carried a penicillin-sensitive strain (bacteriophage type 42 D, 42 F, 1, 2, 7, 8, 9, 10).

Vaccine/toxoid preparation

Six coagulase positive strains of *Staph. aureus* were isolated from older cows in the herd and used for the first batch of vaccine/toxoid in 1955. Subsequent batches were made in the same way using two to six freshly isolated strains each time.

The organisms were grown aerobically at 37° C. for 3 days in 2% dextrose infusion broth for alum precipitation of the toxin. The same strains were grown on trypsin agar slopes for 48 hr., washed off with saline and incubated overnight after formalinizing to 0·4%. The vaccine/toxoid consisted of 50% alum-precipitated toxoid and 50% formalinized whole culture, the latter having been adjusted to contain approximately 3×10^9 organisms per ml. Inoculations were given intramuscularly, as little or no local reaction was found to follow this method of injection.

Vaccination method

In December 1955 twelve first and two second calving cows were inoculated at weekly intervals with 3–5 ml. vaccine/toxoid on four occasions, and each animal was re-vaccinated every 6 months with a further 5 ml. Six first calving cows and one second calver were left as untreated controls.

In subsequent months only first calving cows were brought into the experiment and these were alternately placed in treated and control groups, the former being vaccinated as above. The selection for each group was based solely on the code number each animal was given at calving, cows with even numbers being vaccinated and those with odd numbers left as controls. By February 1957 14 further animals were in the treated group and there were 12 further controls.

The results recorded by May 1958 give data on 25 treated animals and 18 controls, 4 cows having been culled for economic reasons early in the experiment. The work thus covers a period of 2½ years.

Bacteriological and clinical examinations

Every cow was clinically normal when brought into the experiment. Clinical changes were recorded later if a quarter yielded abnormal foremilk prior to milking and/or if the quarter was swollen and inflamed. Strip-cup examination was made twice daily.

Quarter milk-samples from each cow were tested bacteriologically on two occasions prior to vaccination, and every 4–6 weeks afterwards in the following way.

The teat orifice was cleansed with methylated spirit and approximately half an ounce of foremilk drawn from each quarter into McCartney bottles. The samples were stored at 4° C. until the following day when any milk changes were recorded and 0·05 ml. of secretion spread on 10% ox-blood agar plates. The plates were incubated for 20 hr. and all those yielding streptococci and *Staph. aureus* were examined further.

Staphylococci showing any degree of lysis were picked off into broth and coagulase and penicillin sensitivity tests carried out from the broth culture. A 1:3 dilution of human plasma was used for the former test, interpretation being made after 4 hr. tube incubation, and serial broth dilutions used for the sensitivity test. Strains growing in broths containing not less than 0·05 unit penicillin/ml. were recorded as resistant and those growing at lesser strengths recorded as sensitive.

Staphylococci showing very weak plate haemolysis were occasionally isolated, but found to be coagulase negative. In some quarters, however, they appeared to persist as infections of importance.

Eighty-seven isolations of haemolytic staphylococci were phage typed by Mr Davidson at Weybridge and seventy-nine of these classified. No cow was found to be infected with more than one phage type of organism during the trial.

Streptococci were rarely found in either group of cows until July 1956, i.e. 6 months after the trial began. They were mainly *Str. agalactiae*. Five cows yielded streptococci during that month and it was decided from that time to eliminate all streptococcal infections, by local penicillin/streptomycin treatment, from any quarter found to be infected at each monthly survey. Similar local treatment was normally applied to all clinical forms of mastitis, whatever their cause, from the commencement of the experiment. Foremilk samples were automatically taken from such cases prior to treatment and sent to the laboratory for culture, and in this way the incidence and bacteriology of clinical cases was recorded. Clinical samples were cultured similarly to those taken from the groups every 4–6 weeks.

Infections with streptococci or staphylococci were classified as light (1–25 colonies per 0·05 ml. inoculum), medium (26–100 colonies) and heavy (over 100 colonies).

Quarter samples were taken from dry cows during the winter months but not in summer. During July and August local antibiotic treatment of dry udders was given as a prophylactic against summer mastitis.

CLINICAL AND BACTERIOLOGICAL RESULTS

In the vaccinated group the inoculations without doubt exerted some prophylactic effect. Within the first 21 months of the trial only one clinical case of staphylococcal mastitis

occurred in the treated group compared with nine cases in the controls. The establishment of major staphylococcal infections was also considerably influenced by vaccination, as is shown by the fact that there was an incidence of 17 (1·9%) heavy infections in the treated cattle compared with 46 (8·1%) in the uninoculated group.

Table 1 summarizes the position at the end of the first 21 months.

Table 1. *Incidence of staphylococcal infections and clinical quarters during the first* 21 *months*

	Quarter samples tested	Quarters clinical owing to staphylococci	Samples containing *Staph. aureus* (colonies per 0·05 ml. milk)			
			1–25	26–100	Over 100	Total
Treated group	944	1	35 (3·7%)	16 (1·7%)	17 (1·9%)	68 (7%)
Controls	568	9	28 (6%)	19 (3·3%)	46 (8·1%)	93 (16·2%)

During this 21-month period six quarters (7 recordings) of 5 of the 25 cows in the vaccinated group showed clinical mastitis, 1 being staphylococcal, 2 streptococcal, 2 sterile and 1 due to *Corynebacterium pyogenes*. In the control group sixteen quarters (21 recordings) showed clinical mastitis, 9 being staphylococcal, 1 mixed streptococci and staphylococci, 1 streptococci alone and 5 were sterile. Five cows of the control group were sold because of staphylococcal mastitis before April 1957 (nos. 740, 758, 761, 767 and 777) and one cow, no. 765, lost a quarter for the same reason but was retained in the herd.

Many quarters acquired staphylococcal infections which did not become clinical during this 21-month period. This occurred in both groups but many of the infections were transient or mild.

The second phase of the trial, from 21 months to 30 months, is not so encouraging, as a comparison of vaccinated and control groups shows. Cows in the treated group were by this time receiving only a single inoculation of vaccine/toxoid every 6 months and the results suggest that this was insufficient to reinforce the immunity of each animal.

Eight clinical staphylococcal infections appeared in the vaccinated group during this period compared with five in the controls. Heavy infections, however, showed no marked increase in the treated group during this time while in the controls there was still a high incidence.

Table 2 summarizes the over-all picture during the $2\frac{1}{2}$ years.

Table 2. *Incidence of staphylococcal infections and clinical quarters during* $2\frac{1}{2}$ *years*

	Quarter samples tested	Quarters clinical owing to staphylococci	Samples containing *Staph. aureus* (colonies per 0·05 ml. milk)			
			1–25	26–100	Over 100	Total
Treated group	1148	9	78 (6·7%)	31 (2·7%)	30 (2·6%)	139 (12%)
Controls	736	14	49 (6·6%)	31 (4·2%)	67 (9·1%)	147 (19·9%)

Details of the incidence of infections and clinical cases in each cow are given in Tables 3 and 4.

In the vaccinated group (Table 3) heavy infections have occurred in 9 of the 25 cattle, while in the control group (Table 4) they have occurred in 16 out of 18 cattle. The differences in mild and medium infections are not so great, but in some cows in both

groups (e.g. 742, 745, 757, 770, 746 and 715) they were constantly present and very commonly in association with one or more specific quarters. Heavy infections showed a similar quarter predilection, where a number of isolations were made from one cow.

Table 3. *Vaccinated group. Summary of infections following vaccination*

Cow no.	Vaccinations commenced	Staphylococcal infections: Mild	Medium	Heavy	Other infections	Clinicals	Remarks
742	Dec. 1955	8	3	3	5	None	Most infections associated with Whiteside I
743		2	1	1	2	None	—
745		16	3	8	4*	4	Infections associated with Whiteside II and III
747		0	2	0	2	None	Culled Nov. 1957
750		1	3	0	7	2	Quarter lost—*C. pyogenes*
752		1	0	0	0	None	Culled Feb. 1957
753		4	0	0	3†	None	—
754		5	1	4	7	2	Both clinicals staphylococcal, occurring in Jan. 1958
755		1	1	0	4	None	Culled Oct. 1956
756		2	0	0	4	None	—
757		9	8	3	5	2	Both clinicals in Jan. and Feb. 1958—staphylococcal. Most infections Whiteside II and III
762	June 1956	5	1	1	10	1	Clinically streptococcal
764		0	0	0	2	None	—
766		0	0	0	0	None	Culled Aug. 1957
768	Oct. 1956	4	4	2	10	3	Two clinicals streptococcal, 1 staphylococcal (Oct. 1957)
770		9	2	7	3	None	Staphylococcal infections constant in one quarter Whiteside II and III
772		4	0	0	5	1	Died *C. pyogenes mastitis* Aug. 1957
776		0	0	0	12	3	All clinicals—no infection
778		0	0	0	0	None	Culled June 1957
780	Feb. 1957	2	1	0	6†	None	—
782		1	0	1	5†	2	1 clinical streptococcal, 1 nil
784		1	1	0	0	None	—
786		3	0	0	11	None	Unresponsive streptococcal infection. Never clinical
788		0	0	0	0	None	Culled Oct. 1957
792		0	0	0	0	4	4 clinicals—1 streptococcal, 1 staphylococcal, 1 nil
Totals		78	31	30	107	24	Clinicals { 9 staphylococcal; 7 sterile; 6 streptococcal; 2 nil

* Two associated with staphylococcal infections. † One associated with staphylococcal infection.

A further attempt was made to ascertain the importance of heavy, medium and mild infections by a study of the Whiteside reaction on the foremilk of infected and uninfected quarters.

The Whiteside plate test, described by Murphy & Henson (10), using one drop of N-NaOH to five drops of milk was used and the reactions graded negative, I, II or III according to absence or presence and rate of clot formation following stirring. The tests were applied only to samples taken during the last 8 months of the experiment in an effort to assess the importance of staphylococcal infections isolated at each monthly survey. All quarter samples taken from November 1957 were thus checked for Whiteside reaction as well as being cultured, and the results on over 700 samples accumulated.

Comments are entered on Tables 3 and 4 showing the relationship thus found, and it is

significant that cows constantly affected have shown degrees of Whiteside reaction. Even mild infections appear to produce a partial or Whiteside I reaction and heavy infections give rise to Whiteside II or occasionally a grade III reaction; but in all instances the infections must be constantly present in the quarter. Sporadic or transient subclinical infections, whether mild, medium or heavy show no correlation with Whiteside reaction whatsoever.

Table 4. *Control group. Summary of infections*

Cow no.	Vaccinations commenced	Staphylococcal infections			Other infections	Clinicals	Remarks
		Mild	Medium	Heavy			
740	Dec. 1955	6	1	4	12	2	Sold Nov. 1956
746		8	5	13	2	3	Clinicals all staphylococcal, type 42 D
751		7	6	12	10	3	2 clinicals staphylococcal, 1 sterile. Whiteside constantly II or III
758		2	4	5	3	2	Quarter lost. Cow sold Apr. 1957
759		3	5	4	4*	None	Culled Apr. 1957
761		0	0	1	1	1	Staphylococcal. Sold Apr. 1957
763	June 1956	6	0	2	2	None	Sold June 1957
765		3	0	7	8	3	All clinicals staphylococcal
767		3	2	3	3	2	Both clinicals staphylococcal
771	Oct. 1956	3	1	3	3	2	1 clinical staphylococcal
773		3	3	2	0	1	Clinical staphylococcal
775		1	0	0	0	1	—
777		0	0	2	1	2	Sold Apr. 1957. One clinical staphylococcal
779		2	2	2	3	None	—
783	Feb. 1957	0	0	0	2	1	—
785		0	0	3	1	None	—
787		1	1	3	5†	2	Mixed clinical infection
791		0	1	1	1	3	All clinicals sterile
Totals		49	31	67	55	28	Clinicals { 14 staphylococcal / 7 others

* One associated with staphylococcal infection. † Two associated with staphylococcal infections.

DISCUSSION

It is appreciated by the author that the above studies have been carried out on a single herd, but the results obtained might be a useful guide to other workers considering similar experiments. One of the main problems in conducting mastitis trials is the keeping on of control animals, as the susceptibility of individual animals cannot be taken into account. Nevertheless, the selection for vaccination of every second animal to calve was the most reasonable approach that could be made, and accordingly both control and vaccinated groups entered the experiment and were milked and managed in similar circumstances throughout the 2½-year period.

Culling for various reasons including mastitis, upset the number of animals in the control group during the first year, but this has not unduly influenced the results. Total quarter samples taken in both groups are recorded in Tables 1 and 2, and the infection percentages are based on these.

The criterion adopted for recording various degrees of infection has worked very well, but it must be emphasized that transient or sporadic infections of any grade may have no significance. A single herd survey might therefore be misleading unless Whiteside tests are simultaneously carried out on all quarter samples, whether infected or not. A more accurate assessment of the significance of staphylococci in a herd would require a series of tests at weekly or fortnightly intervals, coupling cultural results with Whiteside

reaction. A single major infection may thus cause a Whiteside negative reaction in the milk, while a series of minor infections may lead to Whiteside reactions I or II.

The inclusion of bacterial bodies in the vaccine toxoid used in this trial is probably important. The stimulation of antitoxin response by the inoculation of toxoid only, might not be the factor which will prevent bacterial invasion of a quarter, and it was considered better to study the effect of the vaccine product given under field conditions and to see if protection was given to cows receiving a 'natural' challenge infection.

Spencer and his colleagues (9) have carried out preliminary work using inactivated whole cultures which, they suggest, give better protection than do purified toxoids. In the present trial a number of vaccinated and control cattle were bled from time to time and the value of alpha- and beta-antitoxin responses estimated. Titres of vaccinated cattle at no stage rose higher than 16 alpha or 16 beta units/ml., while in some of the control animals titres as high as 16 alpha units and 32 beta units were obtained. An interesting relationship to udder infections was found here, as in four control cows with high titres, major staphylococcal infections had previously occurred over a period in one or more quarters, and presumably stimulated a blood response. The colonization of the organism in the udder is probably necessary before this response is shown.

The importance of antitoxin titres in the blood stream is therefore confusing and it appears also that some physiological influences play a part in their development. As the cow approaches the dry phase—whether it has been vaccinated or not, or experienced udder infection—the blood serum may show an increase in measurable antitoxin and this in turn is accompanied by the appearance of a milk whey titre.

There is no constant relationship between blood titre and staphylococcal udder infection, neither does it appear from our results that such a titre stimulated by an udder infection gives any protection whatsoever against further bacterial invasion of the udder tissue.

Estimations of the value of bacterial vaccines must at present depend on the results obtained on field work or local challenge with culture. No reliable blood test is available for determining response to such vaccines, and even if there were it might lead to as much confusion as the toxoid/antitoxin relationship to actual immunization against infection.

The phage type of the staphylococci may also be important. In the present trial one type predominated and was of course used in vaccine preparation. The apparent success of vaccination in the earlier part of the experiment might not have resulted if other strains had been used and it is well known that a number of phage types are pathogenic in the bovine udder.

There is obviously room for more study along the lines of the present trial. Staphylococcal infections have assumed a major role in the udder of the cow in recent years and there is an urgent need for any new approach to their control.

SUMMARY

The use of an autogenous vaccine/toxoid in the prophylaxis of clinical and subclinical staphylococcal udder infections in a dairy herd is described. Mainly first calving cows were included in the trial, and the results are given on 25 vaccinated and 18 uninoculated control animals. Four injections of vaccine at weekly intervals were given and every 6 months a further inoculation was given with a view to reinforcing immunity.

Observations were made over a period of $2\frac{1}{2}$ years and there was a significant difference in the number of heavy staphylococcal infections and clinical cases in treated and control groups during the first 18 months, only one clinical case occurring in the treated group compared with 9 in the controls. Major infections totalled 8·1% during this period compared with 1·9% in the treated cattle, total staphylococcal infections being 16·2% compared with 7% in the respective groups.

In the last 12 months of the experiment eight clinical cases occurred in the treated group compared with five in the controls. It is clear from these results compared with those for the first 18 months that the reinforcing doses of vaccine/toxoid every 6 months were insufficient to maintain immunity. Subclinical infections, however, remained at a lower level in the treated group than in the control animals.

The significance of subclinical staphylococcal infections in relation to Whiteside tests is discussed, also the possible relationship of toxoid and bacterial vaccination to actual immunity produced. Some evidence is given that the presence of blood serum antitoxin is not necessarily associated with immunity.

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INFLUENCE OF TEMPERATURE TREATMENT AND SEASON ON THE DILATOMETRIC BEHAVIOUR OF BUTTERFAT*

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(With 3 Figures)

The ratio of solid to liquid butterfat is a major factor influencing the hardness of butter. Two main variables determining this ratio are variations in temperature treatment during the buttermaking process and seasonal variations in the chemical composition of the butterfat.

Results have been reported from this laboratory on studies of the chemical composition of Alberta butterfat (1), and hardness of butter as affected by season and manufacturing method (2). Important hardness differences were observed, and the purpose of this report is to correlate these hardness differences with the proportion of solid fat found by a dilatometric technique.

Conventional buttermaking involves comparatively slow cooling of the fat in the cream, whereas rapid cooling takes place in the chilling units of continuous buttermaking machines. Differences in the rate of cooling of the fat may cause large variations in the hardness of the resulting butter. Two phenomena have been investigated as possible causes. The first is polymorphism, which is the occurrence of unstable crystal modifications. It is the general opinion, however, that polymorphism is of minor importance in fats with a complicated glyceride composition (3), and a recent study (4) revealed no important effects of polymorphism in butterfat. Moreover, polymorphism, if it occurs in butterfat, will be a short-term effect, while the effect of cooling-rate variations is a permanent one. Mixed crystal formation offers a more satisfactory explanation of the differences in crystallization behaviour of slowly and rapidly cooled butterfat (5). Mixed crystal formation involves the inclusion of dissimilar molecules in the crystal lattice and, therefore, results in an increase in the ratio of solid to liquid butterfat.

EXPERIMENTAL PROCEDURE

The dilatometers were of the volumetric type with a capacity of approximately 10 ml. and a stem calibrated in $mm.^3$. The confining fluid was water coloured with a dye, and it was always boiled and cooled before use. Butterfat was prepared from butters of minimum, intermediate, and maximum hardness, obtained in June, September and December respectively (2). The pure fat was prepared from butter by melting, centrifuging and filtering. Before transfer to the dilatometers the filtered fat was dried and de-aerated in round-bottom flasks under reduced pressure (5–10 mm. Hg) for at least 30 min. at 90° C.

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with occasional violent shaking. The dilatometers were weighed before and after filling to obtain the weight of the fat by difference. Two grams of confining fluid were used in all measurements.

The percentages of liquid and solid fat in a mixture may be deduced from knowledge of the thermal expansions of the fat when liquid and solid. The thermal expansion of completely liquid butterfat, measured between 40 and 60° C. was 8·5 mm.^{3}/10 g. ° C. The measurements on completely solid fat were made between −50 and −40° C. and the thermal expansion found to be 5·8 mm.^{3}/10 g. ° C. For these latter measurements a special confining fluid had to be used which consisted of a concentrated solution of either calcium chloride or potassium acetate in alcohol. The thermal expansion of these confining fluids was determined separately. Melting started in all the butterfat samples at about −35° C., and at 0° C. about 20–30 % of the butterfat was liquid, the percentage varying with season, which confirms recent observations (6). The values obtained in the above measurements are the average of at least six determinations and in their calculation corrections were applied for the expansion of the glass.

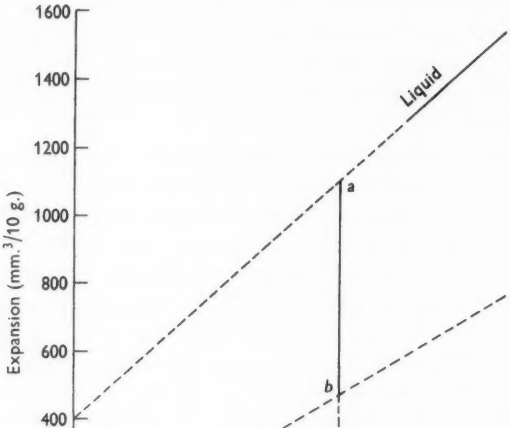

Fig. 1. Curves showing thermal expansion of solid and liquid butterfat (dotted lines are extrapolated), and melting dilation *ab* at 20° C.

When the thermal expansions are represented graphically as in Fig. 1, the melting dilation at any one temperature is equal to the distance between the curves at that temperature (e.g. *ab* in Fig. 1 represents the melting dilation at 20° C.). The melting dilations, i.e. the expansion value for complete isothermal change from solid to liquid, found for the temperatures at which measurements were made, are listed in Table 1.

When the reading for the completely liquid fat is made at 40° C., the melting expansion

$$D_t = \frac{10}{w}(R_{40} - R_t - EW_t) - M_{40,t},$$

where w = weight of butterfat, R_{40}, R_t = readings of dilatometer at 40° and t° C. respectively, EW_t = thermal expansion of 2 g. of water between t° and 40° C., and $M_{40,t}$ = thermal expansion of 10 g. of fat between t° and 40° C. The values for thermal expansion of water were obtained from the International Critical Tables.

Table 1. *Melting dilations of butterfat*

Temperature (° C.)	Melting dilation (mm.³/10 g.)	Temperature (° C.)	Melting dilation (mm.³/10 g.)
10	624	22	656
12	629	24	662
14	635	26	667
16	640	28	673
18	646	30	678
20	651		

The percentage of solid fat in a sample at any one temperature equals the experimentally found melting expansion at that temperature (D_t) divided by the melting dilation at t° C., multiplied by 100.

The technique followed throughout this study was a dynamic one, i.e. the temperature of the bath containing the dilatometers was increased gradually without interruption at the measuring temperatures. The rate of temperature increase of the bath was 10° C. per hour. No complete equilibrium was thus obtained at any one temperature and, therefore, all values reported are to be considered as comparative rather than absolute. However, with the low rate of temperature increase used, the values obtained should not differ greatly from the true solid fat contents.

RESULTS

Influence of cooling treatment. September butterfat only was used for measurements of the effect of cooling treatments. The dilatometers containing the fat were warmed to 45° C., and then cooled in the following ways: (*a*) storage of the dilatometers overnight in air at 15° C.; (*b*) immersion in a 15° C. water-bath; (*c*) immersion in a 10° C. water-bath; (*d*) immersion in a 5° C. water-bath; (*e*) immersion in a 0° C. water-bath. The dilatometers were kept in the water-baths for at least 4 hr. The percentages of solid fat found following these treatments are listed in Table 2. It has been assumed that cooling in water-baths at decreasing temperatures would affect the subsequent melting behaviour mainly, if not entirely, as a result of higher rates of cooling rather than any influence which cooling to different temperatures might have on mixed crystal formation. The solid fat percentage generally increased with increased cooling rate. There was more solid fat at all measuring temperatures when the dilatometers were cooled in water at 15° C. than when they were cooled in air at the same temperature. The solid fat percentage increased with lower cooling temperatures but only within the lower part of the measuring range. Cooling to 5° and to 0° C. in water resulted in very little difference in solid fat content.

When the increments of expansion are plotted against temperature, differential

dilatometric curves (7) are obtained. The curves thus obtained are shown in Figs. 2 and 3. They indicate that the maximum melting of the rapidly chilled fat was between 18° and 20° C., but of the slowly cooled fat it was between 20° and 22° C.

Table 2. *Influence of cooling treatment on the percentage of solid fat in September butterfat*

Temperature of measurement (° C.)	Solid fat % when dilatometers cooled in				
	Air at 15° C.	Water at 15° C.	Water at 10° C.	Water at 5° C.	Water at 0° C.
12	—	—	48	56	56
14	—	—	45	52	53
16	29	36	41	47	47
18	27	34	36	40	39
20	24	27	29	32	30
22	19	21	23	24	23
24	16	19	18	19	18
26	13	15	14	15	15
28	10	12	12	12	12
30	7	9	9	9	9

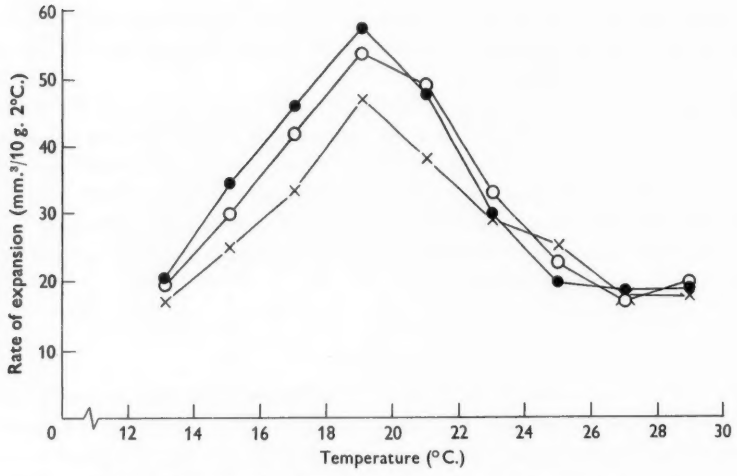

Fig. 2. Influence of cooling treatment on the rate of expansion. ●—●, 0° C.; ○—○, 5° C. ×—×, 10° C.

Influence of seasonal variations. June, September and December butterfats were cooled in two ways, by immersion of the dilatometers in water at 0° C. and by storage of the dilatometers in air at 15° C. The solid fat percentages thus obtained are listed in Table 3. With both treatments there was a considerably higher solids content in December fat than in June fat, while September fat gave intermediate values. The differences were especially marked at the higher measuring temperatures, which indicates variations in the content of higher melting glycerides.

Influence of temperature fluctuations. Butter held at a temperature of 22·5° C. for 1 week maintains a decreased hardness when subsequently held at lower temperatures (2). To determine whether a reduction in solid fat occurs when pure butterfat is similarly treated,

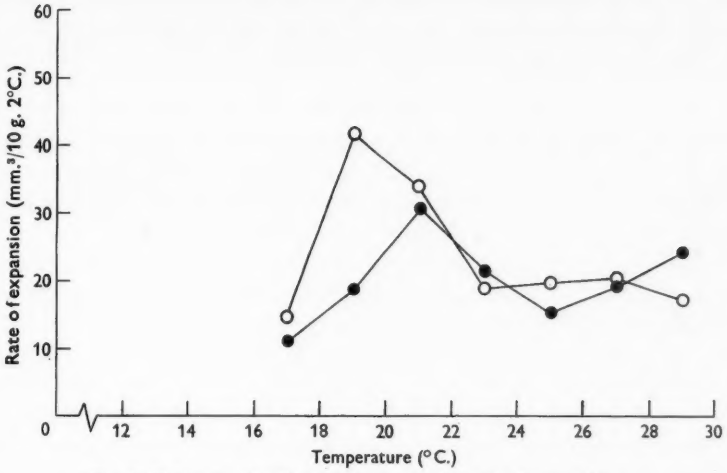

Fig. 3. Influence of cooling treatment on the rate of expansion.
●—●, 15° C. air; ○—○, 15° C. water.

Table 3. *Influence of season on the percentage of solid fat in butterfat*

Temperature of measurement (° C.)	Solid fat (%)					
	Dilatometers cooled in water at 0° C.			Dilatometers cooled in air at 15° C.		
	June	September	December	June	September	December
12	52	56	64	—	—	—
14	48	53	61	—	—	—
16	42	47	56	27	29	36
18	33	39	49	26	27	33
20	25	30	39	23	24	30
22	18	23	30	18	19	24
24	14	18	23	15	16	20
26	11	15	18	10	13	17
28	9	12	15	8	10	15
30	6	9	12	5	7	13

Table 4. *Influence of storage at 22·5° C. on the percentage of solid fat in September butterfat*

Temperature of measurement (° C.)	Solid fat (%)	
	Before storage at 22·5° C.*	After storage at 22·5° C.†
12	56	45
14	53	40
16	47	35
18	39	29
20	30	22
22	23	17
24	18	16
26	15	14
28	12	12
30	9	10

* Dilatometers cooled in water at 0° C.
† After cooling in water at 0° C., dilatometers were stored for 7 days at 22·5° C. and cooled in a refrigerator at 5° C. for 12 hr.

September butterfat was cooled by immersion of the dilatometers in a 0° C. water-bath, subsequently stored at 22·5° C. for 1 week, and finally kept overnight at 5° C. A definite reduction in solid fat content was obtained (Table 4).

DISCUSSION

The thermal expansion of liquid butterfat was found to be higher than that of solid butterfat, an observation that has been reported by several investigators (1,6). Mulder (8), however, found equal values for liquid and solid butterfat, but his values were obtained indirectly from measurements on cream.

Higher cooling rates very markedly increased the solid fat content of the September fat used in these tests, but increases were most apparent at the lower measuring temperatures. It must be assumed that the higher rates of heat transfer, resulting from the lower cooling temperatures, caused the formation of mixed crystals. The fact that there was practically no difference between the values obtained by cooling to 5° and 0° C. indicates that maximum mixed crystal formation must occur at this rate of cooling. It is, therefore, unlikely that a further increase in solid fat content can be obtained with still higher cooling rates (lower cooling temperatures or increased agitation). These results may be of importance in explaining the hardness of continuously made butter which is subjected to a high rate of cooling. Supercooling of the fat will occur when cooling is rapid and the resulting crystals will contain glycerides of different melting points. A fat thus treated will have a lower melting point than a fat which is cooled slowly (9). This is borne out by the curves in Figs. 2 and 3. Maximum melting of the rapidly chilled fat was between 18° and 20° C., but of the slowly cooled fat it was between 20° and 22° C. It has been observed that continuously made butter has a greater oiling-off tendency than conventional butter (10). A lower melting point of the fat in continuously made butter probably is one of the factors responsible for this tendency.

Seasonal differences in solid fat content run parallel to differences in hardness of butter. Maximum, intermediate and minimum values for solid fat content for both rapid and slow cooling, were obtained for December, September and June butterfat respectively. There is an important difference between the seasonal and cooling treatment differences in solid fat content. The seasonal differences indicate relatively large variations in the higher melting glycerides, whereas the cooling treatment variations occur mainly at the lower measuring temperatures, indicating an increase in lower melting-point crystals. It is possible that these two types of crystals may not be equal in their relative effect on butter hardness. Bailey (3) has pointed out that crystals composed in large part of high melting glycerides possess more rigidity and greater stiffening power than crystals of lower melting glycerides. Very slow cooling would result in a minimum of crystals, each of which would have a relatively sharp melting point. It is not likely that this ideal situation will occur in butter made by conventional methods. It must be assumed rather that with that type of butter mixed crystal formation also occurs, though to a much lesser extent than in the continuous process. Evidence for this assumption has been obtained from microscopic studies (11). Once mixed crystals are formed in butterfat, it seems possible, at least at relatively high temperature, to induce recrystallization whereby the solid fat content is permanently decreased. The results in Table 4 show that this treatment resulted in a considerable drop in solid fat content at the lower measuring temperatures. A similar lowering of solid fat content was previously demonstrated microscopically (11).

SUMMARY

The melting dilation of butterfat between 10° and 30° C. has been determined from results obtained for the thermal expansion of solid and liquid butterfat. Measurement of the solid fat percentages obtained with different cooling treatments showed that at higher rates of cooling there was an increase in the solid fat content, which is in accordance with the theory of mixed crystal formation. A cooling rate existed beyond which no further increase in solid fat content took place, since butterfat cooled by immersion in a 5° C. water-bath had practically the same solids content as it had when cooled in a 0° C. water-bath. Differences in cooling rate changed the solid fat content mainly in the region of lower melting-point glycerides. Seasonal variations in solid fat content indicated relatively large differences in the content of higher melting glycerides. There is an indication that not only the quantity of solid fat, but also the composition of the crystals is important as a factor influencing the hardness of butter. It was possible to recrystallize mixed crystals in butterfat at 22·5° C. and thereby lower the solid fat content of the butterfat.

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STEAM DISTILLATION OF TAINTS FROM CREAM

IV. VAPOUR/LIQUID EQUILIBRIUM RELATIONSHIPS FOR MESITYL OXIDE AND SOME α-DIKETONES AS POSSIBLE REFERENCE SUBSTANCES

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(With 7 Figures)

The composition of the tainting substances removed from creams of various types in commercial deodorization equipment has not yet been determined. It can be assumed that these substances will usually be present in only very small proportions, and that among them there will be a wide range of steam-volatilities and, possibly, of butterfat/water solubility distribution coefficients.

Diacetyl and acetoin in low concentrations in water and cream have been used as reference substances for investigations on different types of deodorization apparatus (1, 2, 3, 4, 5). The vapour/liquid equilibrium coefficient for diacetyl in water in concentrations of 1 to 50 p.p.m. at time of partition and at 100° C. has been found to be 39·3 (2). With increase in concentration above 50 p.p.m. the coefficient becomes progressively smaller until at 2000 p.p.m. in the liquid it is 17. The coefficient is lowered by presence of lactose in the solution (in 4·5% lactose solution it is 30), and it is raised by presence of sodium chloride in the solution (in 10% salt solution it is 68) (4).

The coefficient for acetoin in water solutions has been found to be 1·29 over a range of concentrations up to 1·0%. It is not affected by presence of lactose in the solution, but it is raised to 1·70 by the presence of 10% of salt (4).

There is little information available on the vapour/liquid equilibrium relationships for steam volatile substances in very dilute solution. Scott (6) in his investigations on the design of cream treatment equipment has, as part of a working hypothesis, assumed that a vapour/liquid equilibrium coefficient of 10 would be representative of the average properties of the tainting substances in cream, and his computations of the efficiencies of the various types of equipment have been based on this assumption. The primary objective of the present investigation was to find a suitable reference substance with an equilibrium coefficient reasonably close to Scott's assumed value. A secondary objective was to find a suitable substance with a higher butterfat/water solubility distribution coefficient than diacetyl, for an extension of the investigations already reported (3, 4) on the effect of solubility partition equilibrium between butterfat and water on the rate of removal of volatile substances from cream by steam distillation.

A suitable reference substance should have the following properties:

(*a*) It should be easily estimated in very low concentrations, both in water and in cream.

(*b*) It should give a linear relationship between concentration in vapour and concentration in liquid over the range of lower concentrations at which it can be readily estimated, i.e. it should obey Henry's Law over this range of concentrations.

(*c*) It should be innocuous even at higher concentrations than those employed in the investigations, so that where necessary it can be safely used for at least pilot-plant investigations with cream in an experimental butter factory.

Selection of substances for investigation

Mesityl oxide, because of its apparently suitable physical properties and its ready estimation by ultraviolet absorption measurements, was first investigated. For the reasons detailed below, however, it was found to be unsuitable. Attention was therefore turned to a study of the homologues of diacetyl. The reaction between diacetyl and diaminobenzidine hydrochloride to form a coloured quinoxaline compound, used in the Pien, Baisse and Martin method of diacetyl estimation (7), is common to all α-diketones. The reaction permits direct estimation of the diketone in concentrations as low as 0·2 p.p.m. in water, and indirect estimation, i.e. after a preliminary concentration by steam distillation and application of the appropriate correction factor for loss on redistillation, in concentrations lower than 0·2 p.p.m., although for diketones of higher molecular weight the lower limit is somewhat higher than for diacetyl. These substances thus satisfy the above-listed requirement (*a*) for suitable reference substances.

EXPERIMENTAL

The measurements of vapour/liquid equilibrium relationships were made with the continuous vaporization equilibrium still already described (9, 10).

Mesityl oxide was estimated by the ultraviolet absorption technique described by Holman, Lundberg & Burr (11). The α-diketone compounds were estimated by the method of Pien, Baisse & Martin (7) as modified by Cox & Wiley (8).

RESULTS

Mesityl oxide. The sample of mesityl oxide used was prepared by repeated fractionation of a commercial product, giving b.p. 129·5–130° C., n_D^{20} 1·4432 (compare values given by Fuge, Bowden & Jones (12), b.p. 129·9° C./760 mm., n_D^{25} 1·4414).

The results, given in Fig. 1, show that over the range of concentrations from 0·5 to 16 p.p.m. in the liquid at time of partition the relationship between concentration in the vapour and concentration in the liquid was linear. The equilibrium coefficient, read from the slope of the average line, was 74. Since the straight line representing the average vapour/liquid equilibrium relationship did not pass through the origin, it appeared that estimation by means of u.v. absorption at 2400 Å was subject to a constant error even when distilled water was used as the diluent. It seemed likely that in investigations on the deodorization of cream additional interfering substances would be present in the distillate. Mesityl oxide, therefore, was not considered to be a suitable reference substance for the purpose in view.

Acetyl propionyl. The sample used, prepared by fractional distillation of a supply obtained commercially, had b.p. 108° C., d_{20}^{20} 0·9501, n_D^{20} 1·4019 (compare constants given by Heilbron (13), b.p. 108° C., d_4^{19} 0·9565, n_D^{19} 1·40135).

The vapour/liquid equilibrium relationship in aqueous solution, shown in Fig. 2, was found to be linear at concentrations up to 10 p.p.m. in the liquid at time of partition (see Fig. 2A), and over this concentration range the equilibrium coefficient was 51. Over the

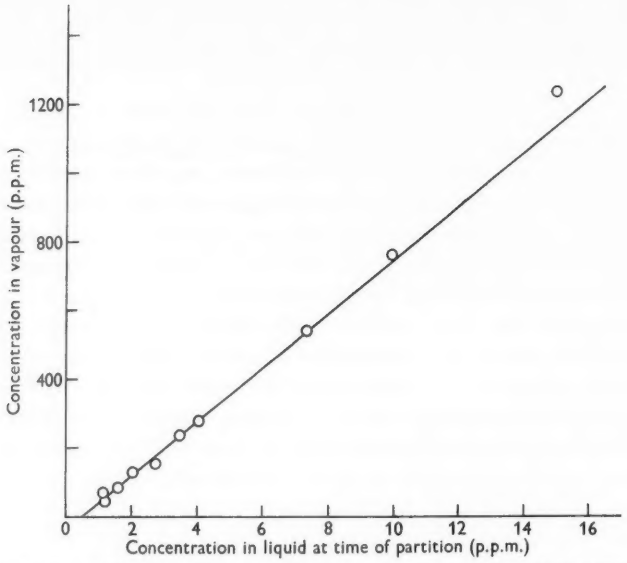

Fig. 1. Vapour/liquid equilibrium relationship for mesityl oxide in dilute aqueous solution at 100° C.

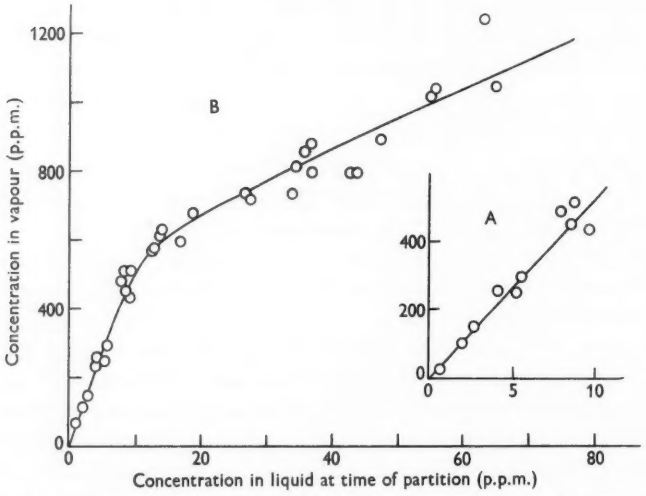

Fig. 2. Vapour/liquid equilibrium relationship for acetylpropionyl in dilute aqueous solution at 100° C.

concentration range 10—20 p.p.m. in the liquid there was a rapid change in the coefficient (see Fig. 2B; also Fig. 7). Above a concentration of 20 p.p.m. in the liquid the relationship appeared again to be linear. At 20 p.p.m. in the liquid the vapour/liquid equilibrium coefficient was 34 and at 40 p.p.m. it was 21·5. Acetyl propionyl was thus found to obey Henry's Law over the range 0–10 p.p.m. in the liquid at time of partition, but at this

concentration the equilibrium coefficient was higher than the desired value of approximately 10.

Acetyl-iso-*butyryl*. The sample, separated from a commercial supply by fractional distillation, had b.p. 116–117° C., d_{20}^{20} 0·9053, n_D^{20} 1·4030 (compare values given by Heilbron (13), b.p. 115–116°, d_{20}^{20} 0·9048).

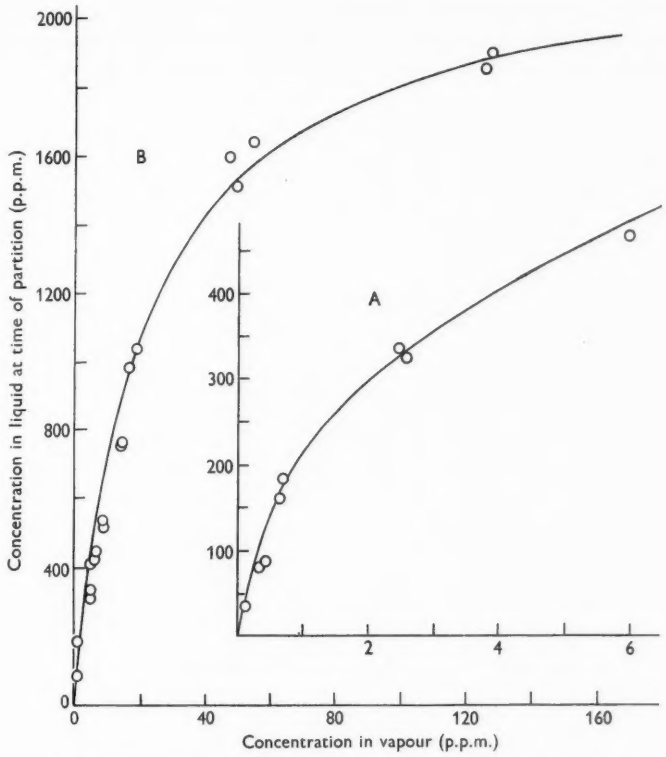

Fig. 3. Vapour/liquid equilibrium relationship for acetyl-*iso*-butyryl in dilute aqueous solution at 100° C.

The results found for the vapour/liquid equilibrium relationship are shown in Fig. 3. The relationship was not linear even at very low concentrations, of the order of 1–3 p.p.m., although there was an approach to linearity at concentrations of 0–1 p.p.m. in the liquid, corresponding to a vapour/liquid equilibrium coefficient of 240 (Fig. 3A). Above 1 p.p.m. in the liquid at time of partition there was a rapid fall in the vapour/liquid equilibrium coefficient. At 6 p.p.m. it was 70, at 60 p.p.m. it was 27, and at 120 p.p.m. it was 16 (see Fig. 7). It was clear that acetyl-*iso*-butyryl was not a reference substance suitable for general application, at any rate for use in water solution.

Acetylvaleryl. The sample, prepared from a commercial product by fractional distillation at atmospheric pressure, had b.p. 147–150° C., d_{20}^{20} 0·8790, n_D^{20} 1·4149.

The results for vapour/liquid equilibrium measurements for the substance in dilute aqueous solution are given in Fig. 4. It is apparent from the curve in Fig. 4B that at

concentrations above 4 p.p.m. in the liquid at time of partition the relationship was approaching the linear. The results, given in Fig. 4A, obtained by the distillation concentration technique used by Pien, Baisse and Martin for diacetyl(7), show that at concentrations between 0·1 and 1 p.p.m. in the liquid the equilibrium relationship was curvilinear. At a concentration of 0·2 p.p.m. in the liquid at time of partition the equilibrium coefficient was of the order of 150 (Fig. 7). Because of the deviations from Henry's Law at low concentrations acetylvaleryl was not considered to be suitable for general use as a reference substance in investigations on cream deodorization.

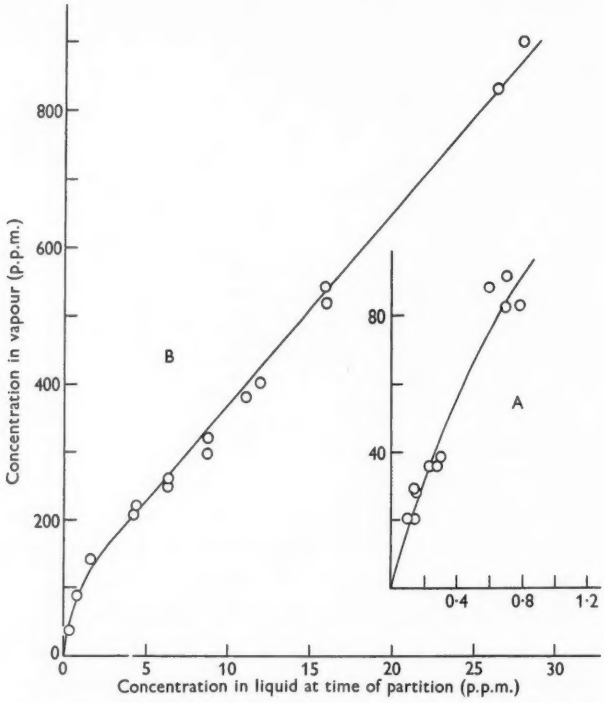

Fig. 4. Vapour/liquid equilibrium relationship for acetylvaleryl in dilute aqueous solution at 100° C.

Acetylcaproyl. The sample, prepared from a commercial product by fractional distillation under reduced pressure, had b.p. 70° C./12 mm., d_{20}^{20} 0·9003, n_D^{20} 1·4237 (compare value quoted by Heilbron(13), b.p. 172–3° C./730 mm.).

The results found for the vapour/liquid equilibrium relationship are shown in Fig. 5. At very low concentrations in the liquid, of the order of 0·1–0·4 p.p.m. (Fig. 5A), the curve connecting concentration in the vapour and concentration in the liquid was linear, representing an equilibrium coefficient of 180. With rise in concentration above 0·5 p.p.m. in the liquid the slope of the curve decreased. At 4 p.p.m. in the liquid the vapour/liquid equilibrium coefficient was approximately 90 and at 8 p.p.m. it was 70. The change in the vapour/liquid equilibrium coefficient with change in concentration in the liquid at time of partition is shown in Fig. 7. Because of the curvilinear nature of the equilibrium

curve and of the high value for the equilibrium coefficient, acetylcaproyl was not considered to be suitable for general use as a reference substance in water solution.

Acetylbenzoyl. The sample used had b.p. 113° C./19 mm. d_{20}^{20} 1·0095, n_D^{20} 1·5330 (compare values quoted by Heilbron(13), b.p. 123° C./23 mm., d_4^{20} 1·0015).

The vapour/liquid equilibrium results are shown in Fig. 6. At a concentration of 0·1 p.p.m. in the liquid the coefficient was found to be 30 (Fig. 6A). With increase in concentration from 0·1 to 0·5 p.p.m. the value of the coefficient fell from 30 to 16, and thereafter with further increase in concentration of acetylbenzoyl in the liquid the change was very gradual. At concentrations above 20 p.p.m. in the liquid the relation between

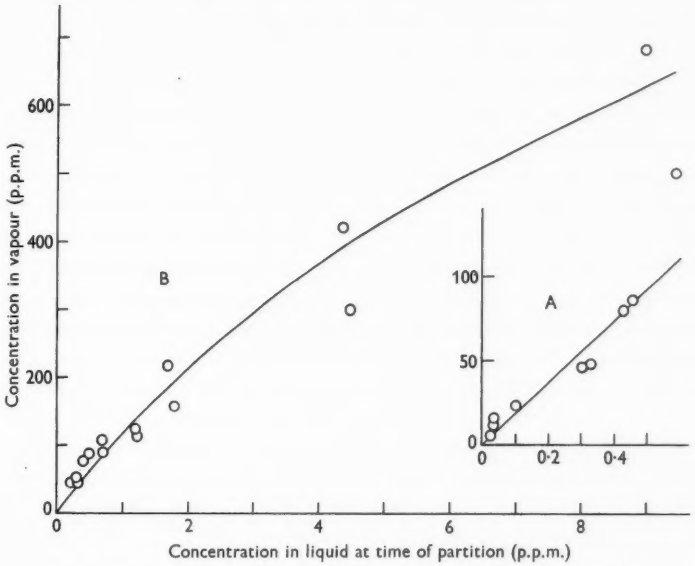

Fig. 5. Vapour/liquid equilibrium relationship for acetylcaproyl in dilute aqueous solution at 100° C.

concentration in the vapour (C_V) and concentration in the liquid (C_L) was almost linear, but because of the initial curvilinear relationship there was still a gradual fall in the vapour/liquid equilibrium coefficient with increase in concentration above 20 p.p.m. in the liquid at time of partition (Fig. 7). At 70 p.p.m. in the liquid the coefficient was 11·5. Because of the greater regularity in the effect of change in concentration in the equilibrium coefficient and of the proximity of the coefficient to the desired value of 10, acetylbenzoyl was considered to be more suitable than any of the other higher diketones for general use as a reference substance.

DISCUSSION

As indicated earlier, it is important that for a compound to be used as a reference substance in cream deodorization investigations the vapour/liquid equilibrium coefficient should be constant over a reasonably wide range of low concentrations. It is evident from Figs. 1–7 that of the substances investigated, apart from mesityl oxide which was rejected because of the likely error of estimation by U.V. absorption on cream distillates, the only

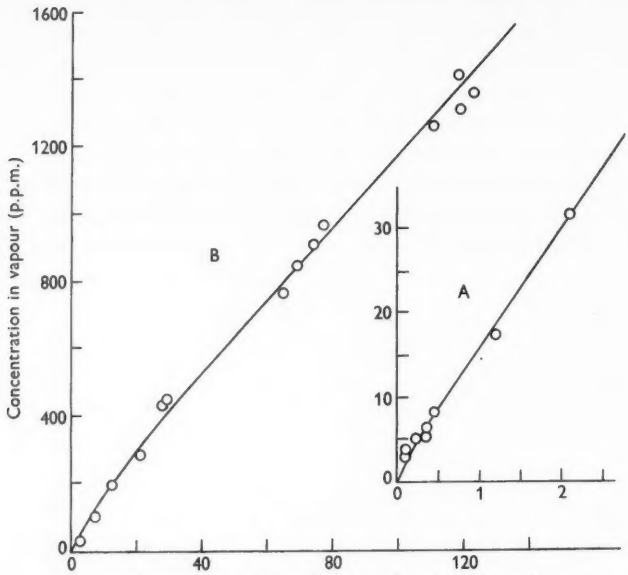

Fig. 6. Vapour/liquid equilibrium relationship for acetylbenzoyl in dilute aqueous solution at 100° C.

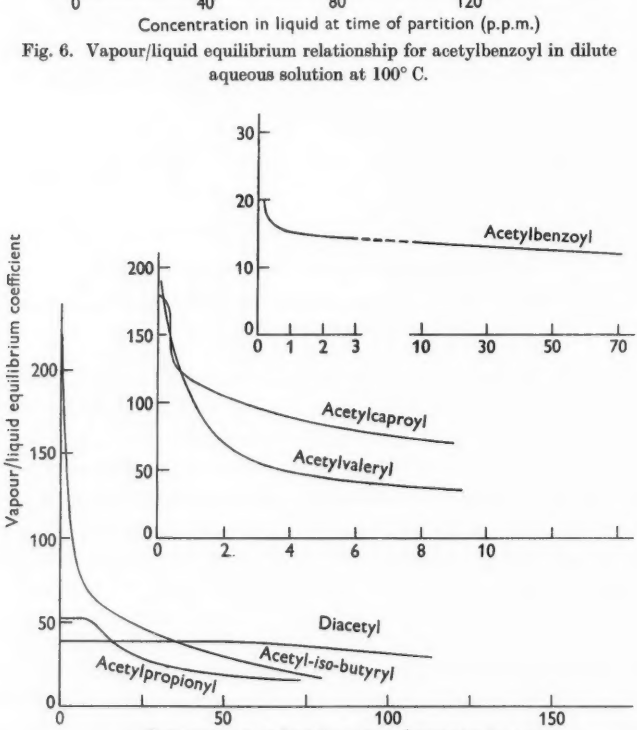

Fig. 7. Change in vapour/liquid equilibrium coefficient with change in concentration for a number of α-diketones in dilute aqueous solution at 100° C.

two fulfilling this requirement over a suitable range of concentrations are diacetyl and acetylpropionyl; and for acetylpropionyl the concentration over which the C_V/C_L relationship is linear is relatively restricted, viz. 0–10 p.p.m. The higher aliphatic α-diketones all have relatively high equilibrium coefficients when the concentrations in the liquid at time of partition are very low, and for acetylbenzoyl there are some variations with change in concentration. The investigation therefore did not provide data which would permit selection of a reference substance more suitable than diacetyl and acetoin previously employed.

For the marked divergence of the α-diketones from Henry's Law at low concentrations, in contrast with the close conformity with this law shown by acetoin and mesityl oxide, the author has no experimentally based explanation to offer. The α-diketones are known to enolize to some extent, and the enol-form is likely to be less steam volatile than the ketoform. But in pure diacetyl the proportion of enol-form is not greater than 1·1% (Schwarzenbach & Wittwer (14), Gero (15)), and the proportion of enol-form appears likely to decrease with increase in extent of dilution with water. Unless the keto-enol equilibrium is greatly altered towards increase in content of enol-form with rise in temperature to 100° C. it does not seem likely that the change in vapour/liquid equilibrium relationships with change in concentration of diketone in the liquid at time of partition can derive from the effect of change in concentration on the keto-enol equilibrium. It is possible, however, that because of the labile nature of the diketones the vapour/liquid equilibrium relationships at high dilution are not widely representative for steam volatile substances. Hence, while the results submitted show that removal of some substances by steam distillation from water solution becomes relatively easier at an increasing dilution, it does not follow that this would apply to substances less labile than the α-diketones.

Although the higher α-diketones investigated were found not to have the full range of properties required for a reference substance for detailed study of the operation of deodorization equipment, they have been found useful in investigations on the effect of the butterfat/water solubility distribution coefficient on the vapour/liquid equilibrium coefficient for cream (see Paper VII in this series), and in a study of the operation of a thin layer countercurrent deodorizer (see Paper IX in this series).

SUMMARY

1. Measurements, by means of a continuous vaporization equilibrium still, of vapour/liquid equilibrium relationships in the steam distillation of dilute aqueous solutions showed that for mesityl oxide the relationship was linear, equilibrium coefficient $(C_V/C_L)=74$.

2. For acetylpropionyl it was linear over the concentration range 1–10 p.p.m. in the liquid, $C_V/C_L=51$, and above a concentration of 20 p.p.m. it was again approximately linear. At 20 and 50 p.p.m. in the liquid the values found for C_V/C_L were 34 and 21·5 respectively.

3. For acetyl-*iso*-butyryl it was not linear even at very low concentrations, of the order of 1–3 p.p.m. in the liquid, but there was an approach to linearity at concentrations 0–1 p.p.m., $C_V/C_L=240$. At concentrations above 1 p.p.m. in the liquid there was a steady fall in the value for the equilibrium coefficient.

4. For acetylvaleryl it was curvilinear even over the concentration range 0·1–4·0 p.p.m.

in the liquid. At 0·5–1·0 p.p.m. in the liquid the equilibrium coefficient was of the order of 240. Above a concentration of 4 p.p.m. in the liquid the relationship was linear.

5. For acetylcaproyl it was approximately linear only over the concentration 0·1–0·4 p.p.m. in the liquid, $C_V/C_L = 180$. At 4 and 8 p.p.m. in the liquid the coefficients were 90 and 70 respectively.

6. For acetylbenzoyl it was curvilinear at low concentrations, i.e. 0·1–0·5 p.p.m. in the liquid, $C_V/C_L = 30$ decreasing to 16. Above this concentration there was a gradual fall in the equilibrium coefficient to 11·5 at 70 p.p.m. in the liquid.

7. Of the substances investigated only diacetyl and acetylpropionyl and, within limits, acetylbenzoyl were considered to be of suitable properties for use as reference substances in detailed investigations on cream deodorization equipment.

The thanks of the author are due to Mr E. R. Elley, who did the analytical work.

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STEAM DISTILLATION OF TAINTS FROM CREAM

V. VAPOUR/LIQUID EQUILIBRIUM RELATIONSHIPS FOR SOME α-DIKETONES IN LACTOSE SOLUTION

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(With 5 Figures)

Vapour/liquid equilibrium relationships for some steam volatile substances, mainly α-diketones, have been discussed in previous papers (1, 2, 3, 4). Most of the diketones studied did not obey Henry's Law over the required range of concentrations, and therefore were not considered suitable for general use in studies on cream deodorization equipment (4). They have proved useful, however, in investigations on the rates of removal of substances of different butterfat/water distribution coefficients from cream (see Paper VII of this series). It has already been shown (3) that the vapour/liquid equilibrium coefficient for diacetyl is lowered by presence of lactose in solution. Before the other diketones could be used satisfactorily as reference substances in cream it was necessary to determine the effect of lactose on their steam-distillation properties.

In cream as it reaches the deodorization equipment there is usually some diluent water from condensed steam, which would reduce the lactose content in the cream plasma from the 5·0% average. In the present investigation, therefore, the measurements have been made mainly on a 4·5% lactose solution, although for some of the substances the comparison was made over a range of lactose concentrations.

EXPERIMENTAL

The diketones were purified,* and vapour/liquid equilibrium coefficients were measured, as already described for water solutions (4). In the estimation of the diketones by the method of Pien, Baisse & Martin (5) allowance was made for the effect of lactose in solution on the development of the quinoxaline colour.

With preheating to 85–90° C. in the continuous vaporization equilibrium apparatus (6) the extent of dilution of the lactose solution by condensed steam after passage through the heavily lagged uptake-tube was not appreciable. In the study of the results, therefore, it has been assumed that the lactose concentrations in the liquid at time of partition were the same as those in the original solution.

RESULTS

Acetylpropionyl in 4·5% lactose solution. The results of two runs in the continuous vaporization equilibrium still with acetylpropionyl in 4·5% lactose solutions over the concentration range 0–12 p.p.m. in the liquid at time of partition are shown in Fig. 1.

* Analysis of the selected fractions of the diketones by gas chromatography indicated that they were all of a satisfactory degree of purity.

The relationship was linear over the whole of this concentration range. The vapour/liquid equilibrium coefficient for the average line was 51, i.e. the same as found for water over the same range of concentrations (4).

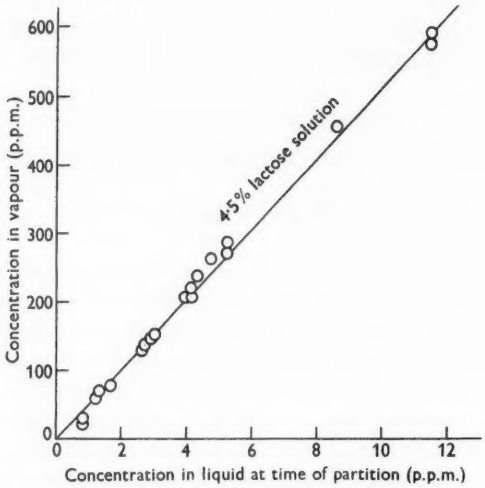

Fig. 1. Vapour/liquid equilibrium relationship for acetylpropionyl in 4·5% lactose solution.

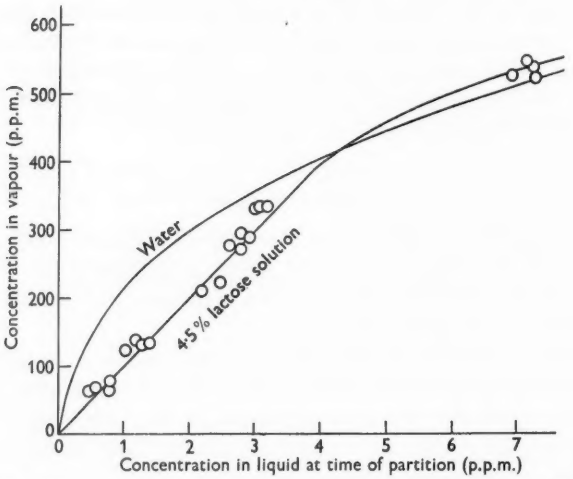

Fig. 2. Vapour/liquid equilibrium relationship for acetyl-*iso*-butyryl in 4·5% lactose solution compared with relationship previously found for the diketone in water solution.

Acetyl-iso-*butyryl in lactose solution*. The results are given in Fig. 2. At concentrations up to 3·5 p.p.m. in the liquid at time of partition the relationship was linear, the equilibrium coefficient being 100. With rise in concentration above this value there was a fall in the vapour/liquid equilibrium coefficient. Comparison with the corresponding curve for acetyl-*iso*-butyryl in water solution (3) (given also in Fig. 2) shows that at concentrations

up to about 4 p.p.m. in the liquid at time of partition the coefficient was lowered by presence of 4·5% of lactose in the solution. Above this concentration over the range of concentrations studied the difference between the results for water and lactose solution was not greater than the possible error of the methods used.

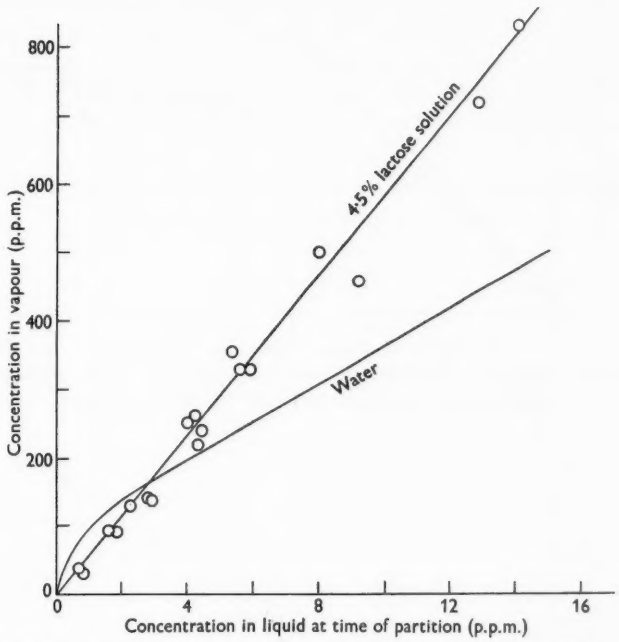

Fig. 3. Vapour/liquid equilibrium relationship for acetylvaleryl in 4·5% lactose solution compared with relationship previously found for the diketone in water solution.

Acetylvaleryl. The results in Fig. 3 show that the vapour/liquid equilibrium relationship in 4·5% lactose solution was linear up to a concentration of 15 p.p.m. in the liquid, and the equilibrium coefficient was 59. Comparison with the corresponding curve for water (4) (given also in Fig. 3) shows that the effect of lactose was to decrease the coefficient up to a concentration of 3 p.p.m. in the liquid, and above this concentration to increase it. At a concentration of 10 p.p.m. in the liquid the coefficient was raised by presence of lactose from 37 to 59.

Acetylcaproyl. The results given in Fig. 4 show that over the range of concentrations investigated, viz. up to 8 p.p.m. in the liquid at time of partition, the effect of lactose in 4·5% solution was to lower the vapour/liquid equilibrium coefficient below the corresponding value for solution in water (4). At 4 p.p.m. in the liquid the coefficient in lactose solution was 58 compared with 94 in water.

Acetylbenzoyl. The results in Fig. 5 show that the vapour/liquid equilibrium relationship in 4·5% lactose solution was linear up to concentrations of at least 10 p.p.m. in the liquid at time of partition. The coefficient read from the average line was 13·7. At the lower concentrations the coefficient was somewhat lower than for the diketone in water solution (4).

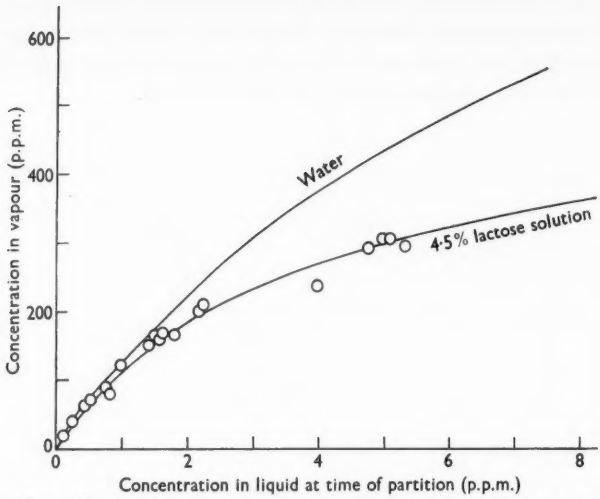

Fig. 4. Vapour/liquid equilibrium relationship for acetylcaproyl in 4·5% lactose solution compared with relationship previously found for the diketone in water solution.

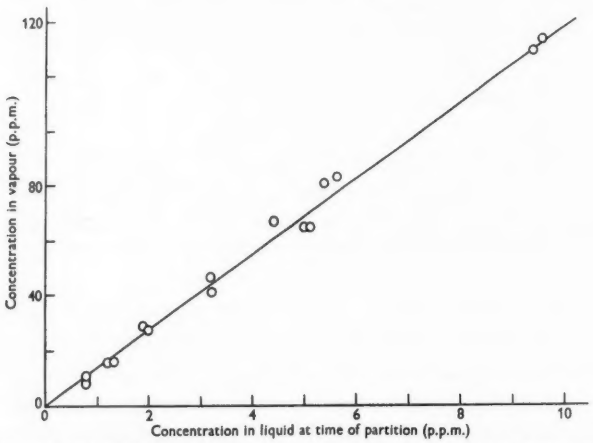

Fig. 5. Vapour/liquid equilibrium relationship for acetylbenzoyl in 4·5% lactose solution.

Effect of concentration of lactose on the vapour/liquid equilibrium coefficients of the diketones. The vapour/liquid equilibrium relationships for these substances at varying concentrations in water solutions has been shown to be mainly curvilinear (3). A direct graphical comparison of the values for the equilibrium coefficients in water with those in lactose solutions of various concentrations would be possible only if the results could be obtained for a uniform concentration of the substance in the liquid at time of partition. With varying effect of the lactose on the equilibrium coefficient it is difficult to arrange the initial concentrations of the reference substance to give this uniform final concentration in the liquid at equilibrium. The information could have been obtained by means of a series of runs in the equilibrium still, each run with one concentration of lactose and

varying concentrations of reference substance. From the curves so obtained a series of comparative graphs could have been deduced. Since, however, it was the object of the present investigation to obtain indicative rather than definitive results, the work has not been extended in this way.

In Table 1 the results for the equilibrium coefficients of the diketones in solutions containing various concentrations of lactose are compared with the coefficients for the same concentrations of diketone in water solution, read from the graphs presented in Paper IV of this series. It is apparent from the table that the effect of change in concentration of lactose was not uniform for all the diketones investigated.

Table 1. *Effect of lactose solution on the vapour/liquid equilibrium coefficients for a number of α-diketones*

Reference substance	Lactose concentration (%)	Concn. of reference substance in solution at time of partition (p.p.m.)	Equilibrium coefficient	
			In lactose solution	In similar concentration in water
Acetylpropionyl	0·2	3·2	55	51
	0·5	2·7	53	51
	1·0	2·8	52	51
	2·5	2·9	51	51
	5·0	3·2	49	51
	10·0	3·9	44	51
Acetyl-*iso*-butyryl	1	0·4	203	300
	3	0·7	103	260
	10	1·7	41	168
Acetylvaleryl	0·5	5·5	80	45
	1·0	5·0	80	46
	2·5	5·1	77	46
	5	5·5	61	43
	10	6·3	53	42
Acetylcaproyl	0·2	1·8	188	112
	0·5	1·7	182	110
	1·0	1·8	155	101
	2·5	2·0	138	102
	5·0	2·5	95	101
	10·0	2·9	89	99
Acetylbenzoyl	1	4·0	14·0	14·2
	2·5	4·9	13·5	14·0
	5·0	4·4	13·0	14·2
	10·0	5·3	13·8	14·0

For acetylpropionyl it has already been shown (Fig. 1) that the coefficient is not appreciably affected by presence of 4·5% lactose in the solution. The results in Table 1 indicate that the coefficient was lower in 10% lactose solution than in water solution.

For acetyl-*iso*-butyryl over the concentration range 0·4–1·7 p.p.m. in the liquid at time of partition there was a reduction in the coefficient at all lactose concentrations over the range 1–10%.

For acetylvaleryl at a concentration of 5–6 p.p.m. in the liquid at time of partition there was an increase in the coefficient at all lactose concentrations over the range 0·5–10%.

For acetylcaproyl over the concentration range 1·2–2·9 p.p.m. in the liquid at time of partition there was an increase in the coefficient in lactose solution up to 2·5% concentration, and a decrease in lactose solution of 5 and 10% concentrations.

For acetylbenzoyl at 4–5 p.p.m. in the liquid at time of partition there was little effect on the coefficient for lactose solutions of all concentrations over the range 1–10 p.p.m. (compare the results with those in Fig. 5).

DISCUSSION

There was no uniformity in the effect of lactose in solution on the vapour/liquid equilibrium relationships for the different α-diketones. Study of the reasons for these differences, however, was not within the scope of the present investigation. The results provide data necessary for a comparison of the vapour/liquid equilibrium relationships in single-phase aqueous lactose solution with the relationships in the two-phase butterfat/cream-plasma solution (see Paper VII of this series).

SUMMARY

1. The effect of 4·5% lactose in solution on the vapour/liquid equilibrium relationship for acetylpropionyl, acetyl-*iso*-butyryl, acetylvaleryl, acetylcaproyl and acetylbenzoyl has been investigated.

2. There was no appreciable effect on the relationship for acetylpropionyl and acetylbenzoyl.

3. For acetyl-*iso*-butyryl there was a lowering of the coefficient at low concentration of diketone in the liquid, but at concentrations above 4 p.p.m. there was no appreciable effect.

4. For acetylvaleryl at low concentrations in the liquid the coefficient was lowered, and at concentrations above 3·0 p.p.m. in the liquid it was increased.

5. For acetylcaproyl the coefficient was lowered by presence of 4·5% lactose in the solution.

6. Change in concentration of lactose over the range 0·5–10% had little effect on the vapour/liquid equilibrium coefficient for acetylpropionyl and acetylbenzoyl, but it caused a progressively increased reduction in the coefficient for acetyl-*iso*-butyryl, acetylvaleryl and acetylcaproyl.

The author is indebted to Mr E. R. Elley for skilled assistance with the analytical work, and to Dr E. L. Richards for the examination of the diketone samples by gas chromatography.

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STEAM DISTILLATION OF TAINTS FROM CREAM.

VI. BUTTERFAT/LACTOSE-SOLUTION DISTRIBUTION COEFFICIENTS OF SOME REFERENCE SUBSTANCES

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(With 3 Figures)

The vapour pressure of volatile tainting substances in cream is related primarily to the concentration of the substance in the aqueous portion of the cream ((1), p. 325). For any given concentration of the taint in a cream of given fat content, the concentration in the plasma is dependent on the distribution coefficient between butterfat and cream plasma for the particular substance at the temperature obtaining (2). Since the tainting substances normally present in commercial cream have not been identified, it is necessary in the meantime in investigations on this subject to use appropriate reference substances for study of the influence of different factors on the effectiveness of cream deodorization equipment (1, 3).

Distribution equilibrium relationships have been extensively studied in connexion with investigations on the purification or isolation of substances by multiple and counter-current extraction processes (4). There is, however, little information on these relationships for substances in very dilute solution. For the two substances earlier used as reference substances, acetoin and diacetyl, the butterfat/water distribution coefficients were found to be relatively small (1), and therefore the effect of presence of butterfat in the cream on the vapour/liquid equilibrium coefficient was not of major significance (2,5). It seemed likely that the distribution coefficients for the higher diketones would be greater, and that therefore they would cause a more important change in the vapour/liquid equilibrium relationship for cream as compared with water or lactose solutions.

In cream deodorization equipment the vapour/liquid equilibrium of the tainting substances between steam and cream is normally established at elevated temperatures, although in most types of apparatus there is provision for application of some degree of reduced pressure. In the Vacreator vacuum-pasteurizer of the design that has been in common use for upwards of 30 years (6) the main deodorization occurs at 6–15 in. of mercury reduction in pressure; but in the final, or flash-cooling, section, the partition of the substances between steam and cream takes place at a relatively high vacuum, viz. 26–28 in. of mercury, corresponding to temperatures of 126–101° F. In some types of equipment the only steam available as carrier vapour to effect the deodorization is obtained by flash ebullition as the result of transfer of the cream to a region of lower pressure. The quantity of deodorization steam produced is then directly related to the pressure change, and the lower the temperature of deodorization the greater is the quantity of carrier vapour released. Since the concentration of the tainting substance in the vapour is directly proportional to the concentration in the aqueous phase at time of partition, the effect of change of temperature on the solubility distribution equilibrium of

a substance between butterfat and cream plasma can be expected to influence the efficiency of taint removal from the cream at the temperature of steam distillation applied. Reference has been made to this aspect in previous papers in this series ((1), p. 325; (5), pp. 54–6).

Some results of direct measurement of the butterfat/water distribution coefficients for diacetyl and acetoin at 40° and 60° C. have already been presented ((1), p. 325). These measurements were made using a centrifuge for separation of the mixture into two layers. In the centrifugal method, however, there is difficulty in maintaining the temperature during the centrifuging, and the results are somewhat unreliable. It was found that with care to avoid vigorous agitation a satisfactory separation of a reasonably clear aqueous layer could be obtained without centrifuging. It was then possible to ensure separation at approximately the desired temperature. When the substance under investigation has a high vapour/liquid equilibrium coefficient, however, there is at high temperatures danger of loss in vapour form during separation of the two phases. Measurements by the direct separation method were therefore done only at 40° and 60° C. Since the aqueous phase in cream is a slightly diluted skim-milk, the measurements of distribution coefficient by the direct method were made with 4·5% lactose solutions in contact with the butterfat (7).

In milk the butterfat is present as globules of average diameter 3–5 μ. Because of the fineness of this dispersion of butterfat, it may be assumed that in milk the distribution equilibrium of a substance between butterfat and plasma is rapidly established. Based on this assumption an indirect method was utilized for measuring the coefficient of distribution of some of the reference substances between butterfat and skim-milk, and the effect of change in temperature on this coefficient. It required use of a plate pasteurizer for continuously heating whole milk to the desired temperature and an hermetic cream separator for separating the preheated milk into cream and skim-milk. Estimations by this method were made only with acetoin, diacetyl and acetylpropionyl, other diketones not being available in sufficient quantity for investigation by this method.

EXPERIMENTAL

Measurement of butterfat/water distribution coefficient

(*a*) *Direct method.* Butterfat (50 g.) and 4·5% lactose solution (50 g.) were placed in each of two conical separating funnels with short outlet tubes, and the substance under investigation was added in aqueous solution to give a suitable over-all concentration. The actual concentration used was varied with variation in the distribution coefficient for the substance under investigation, to give a suitable concentration in the aqueous phase for direct colorimetric estimation. The over-all concentrations ranged from 2 to 250 p.p.m. One of the funnels was placed in a water-bath at 40° C. and the other in a water-bath at 60° C. They were allowed to stand for 30 min. to permit heating of the contents to bath temperature and were then inverted several times at frequent intervals for 30 min. For substances with a very high distribution coefficient in favour of the butterfat it was an advantage to add only a portion of the lactose solution to begin with, and after mixing to stand at the desired temperature for some time to permit approximate establishment of equilibrium before adding the remainder of the water (establishment of the final equilibrium is more rapid when the substance is required to diffuse from the more con-

centrated to the more dilute solution). The funnels were finally allowed to stand in the water-bath until the aqueous layer had reached maximum clarity, complete clarity being seldom obtained. The diketone was estimated directly in the aqueous layer by use of the diaminobenzidine reagent of Pien, Baisse & Martin (8). The concentration in the butterfat layer was calculated by difference, on the assumption that there was no loss during the period of establishment of equilibrium. The butterfat/water distribution coefficient was derived from the results for the concentrations in the two phases at equilibrium.

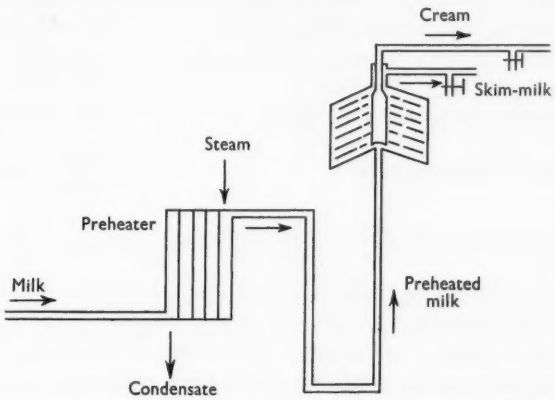

Fig. 1. Apparatus for indirect method of estimating distribution coefficients.

(*b*) *Indirect method.* A 660 gal./hr. Alfa-Laval hermetic cream separator was connected by a $1\frac{1}{2}$ in. diameter pipe, 15 ft. long, to a plate preheater. The cream and skim-milk outlets from the separator were fitted with side-tubes and milking-machine dropper-taps, and the tap tubulures were fitted with rubber bungs to take the neck of a 160 ml. sample bottle (Fig. 1). Whole milk of good quality was pasteurized and at the same time cleared relatively free from naturally occurring diacetyl by treatment at high temperature and high intensity in a Vacreator vacuum-pasteurizer. Pasteurization was necessary to eliminate bacteria that otherwise might have destroyed diacetyl or other α-diketone used as reference substance. The reference substance was added to the cooled pasteurized milk to give the required concentration. The milk was then pumped at constant rate through the plate heat-exchanger, where it was raised by direct steam to the required temperature. The heated milk was delivered to the separator at about 300 gal./hr., giving a holding time of 13–14 sec. at the final temperature. The outlet control valves were adjusted to give a cream of about 35% fat content. Samples of the cream and skim-milk were taken simultaneously through the sampling cocks into bottles fitted tightly on to the rubber stoppers on the tubulures, thus avoiding loss of reference substance from the hot liquid to the atmosphere during sampling. The bore of the tubulures was large enough to permit gravity flow of the sample into the bottle by direct displacement of air.

By varying the temperature of preheating a series of samples was obtained representing the equilibrium conditions over the whole required range of temperatures, without interruption of delivery of milk from the vat.

Butterfat was estimated in the milk samples by the Gerber method, in the cream samples by the Babcock method, and in the skim-milk samples by the Roese–Gottlieb

method. Diketones were estimated in the skim-milk and in the cream by the method of Pien *et al.* (8).

The fat contents of the skim-milk were uniformly low, of the order of 0·06–0·08%. The effect of this fat on the distribution coefficient was therefore not taken into account in the calculations. The concentration of diketone in the fat, and hence the distribution coefficient, was computed as in the following example:

% fat and % plasma in cream by estimation	= 37 and 63
Concentration of diketone in skim-milk by estimation	= 0·87 p.p.m.
Wt. of diketone in the plasma of 100 g. cream	$= 63 \times 0{\cdot}87 = 55\,\mu g.$
Concentration of diketone in cream by estimation	= 1·2 p.p.m.
Wt. of diketone in 100 g. of cream	$= 100 \times 1{\cdot}2 = 120\,\mu g.$
Wt. of diketone in fat in 100 g. of cream	$= 120 - 55 = 65\,\mu g.$
Concentration of diketone in fat	$= \frac{65\,\mu g.}{37\ g.} = 1{\cdot}7$ p.p.m.
Hence butterfat/skim-milk distribution coefficient	$= \frac{1{\cdot}7}{0{\cdot}87} = 2{\cdot}0$

The method was subject to considerable error because of error of colorimetric estimations of diketone; moreover, an error in estimation of diketone in the skim-milk led to an error in the opposite direction in the result for content of diketone in the fat. The error thus had a double effect on the result for distribution coefficient. In addition, for substances with a high distribution coefficient, a small error in the estimation of concentration in the liquid introduces a large error into the final experimental value for the coefficient. The results, however, were considered to be sufficiently accurate for the purposes of the present investigation, which is designed to formulate general principles rather than to provide standard values for the characteristics of the diketones used.

RESULTS

Distribution coefficients

(*a*) *Direct method.* The results for estimations on acetoin and a number of α-diketones at 40° and 60° C. are given in Table 1. Except for acetoin and acetylbenzoyl there was an appreciable rise in the coefficient with rise in temperature from 40° to 60° C.

Table 1. *Effect of temperature on the coefficients of distribution between butterfat and 4·5% lactose solution*

(Estimations by direct method)

Substance	Distribution coefficients at 40° C. (103·7° F.)	60° C. (140° F.)	100° C. (by extrapolation)
Acetoin*	0·18	0·19	0·21
Diacetyl	0·67	0·89	1·33
Acetylpropionyl	2·3	3·3	5·3
Acetyl-*iso*-butyryl	11·3	14·3	20·3
Acetylvaleryl	17·4	19·4	23·4
Acetylcaproyl	110	124	152
Acetylbenzoyl	51	50	48

* Results for water solution as quoted in a), p. 325.

(*b*) *Indirect method.* Results for acetoin and diacetyl are given in Fig. 2 and for acetylpropionyl in Fig. 3. The distribution-coefficient/temperature relationship was linear for all three substances, and for all three there was a rise in distribution coefficient with rise in temperature. For acetoin the change between 85° and 200° F. was from 0·19 to 0·25

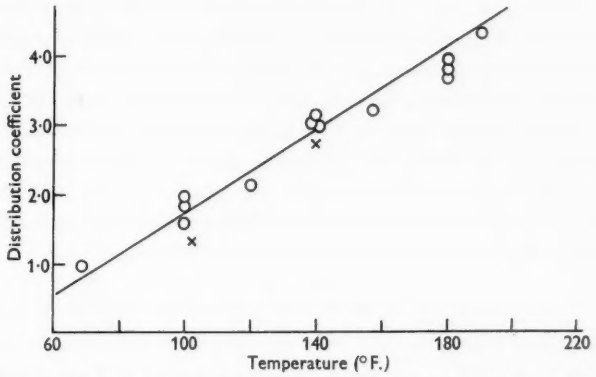

Fig. 2. Effect of temperature on coefficients of distribution of acetoin and diacetyl between butterfat and skim-milk. (Points marked × represent results obtained by direct method.)

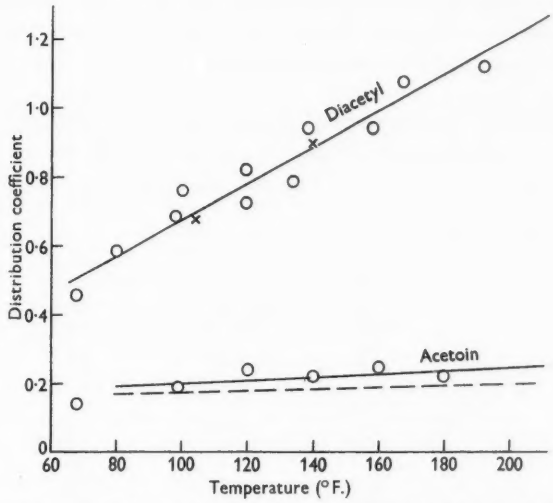

Fig. 3. Effect of temperature on coefficient of distribution of acetylpropionyl between butterfat and skim-milk. (Points marked × represent results obtained by direct method.)

and the extrapolated value at 212° F. was 0·255; for diacetyl the corresponding figures were 0·6–1·2, and 1·27; and for acetylpropionyl they were 2·3–5·6, and 5·9. The results obtained by the direct method for the coefficient of distribution of diacetyl and acetylpropionyl between butterfat and 4·5% lactose solution, shown as a cross in Figs. 2 and 3, were in reasonably good agreement with those obtained by the indirect method for the coefficients between butterfat and skim-milk.

Distribution coefficients for acetyl-iso-butyryl, acetylvaleryl, acetylcaproyl and acetylbenzoyl at 100° C.

In the continuous vaporization equilibrium still[9,10] the vapour/liquid equilibrium coefficients are measured at the prevailing atmospheric pressure, which at this Institute corresponds to a boiling-point for water of approximately 100° C. To permit computation of the expected vapour/liquid equilibrium relationship for a reference substance in cream, therefore, it was necessary to know the distribution coefficient at 100° C. For acetoin, diacetyl and acetylpropionyl this was read by extrapolation from the average lines in Figs. 2 and 3. For the higher diketones under investigation it was computed by extrapolation to this temperature from the experimental results obtained by the direct method at 40° and 60° C. (Table 1), on the assumption that the distribution-coefficient/temperature relationships were linear as was found for diacetyl and acetylpropionyl (Figs. 2, 3). The extrapolated values are shown in the last column of Table 1.

Effect of concentration of reference substance on the distribution coefficient

The results shown in Figs. 2 and 3 for diacetyl and acetylpropionyl were obtained with concentrations of 1 and 10 p.p.m. of reference substance in the original milk. From the close grouping of the points around the average line it is apparent that over this range of concentration there was no change in magnitude of the distribution coefficient with change in concentration. Results obtained by the indirect method over a wider range of concentrations are given in Table 2. At both temperatures the coefficients were constant within the range of accuracy of the method used.

Table 2. *Effect of concentrations of reference substance on butterfat/skim-milk distribution coefficients at* 112° F. *and* 180° F.

	Temperature of partition equilibrium			
	112° F.		180° F.	
Diketone	Concn. in skim-milk at time of partition	Distribution coefficient	Concn. in skim-milk at time of partition	Distribution coefficient
Diacetyl	0·9	0·67	0·8	1·15
	2·2	0·82	2·2	1·05
	4·2	0·75	4·0	1·05
	8·2	0·78	7·4	1·29
	18·4	0·61	15·0	1·14
	35·5	0·71	21·0	1·25
	41·0	0·83	28·5	1·29
	61·0	0·65	43·0	1·09
	76·0	0·68	54·0	1·40
	142·0	0·66	66·0	1·41
	210·0	0·77	120·0	1·31
	—	—	204·0	1·29
Acetylpropionyl	0·7	3·49	0·66	4·41
	1·4	3·15	1·26	4·71
	2·6	3·37	1·35	5·31
	4·5	3·55	4·2	4·85
	6·5	3·12	6·1	4·69
	9·8	3·50	8·8	4·45
	10·5	3·10	10·0	4·26
	25·5	3·21	21·0	5·02
	36·0	3·26	32·0	5·16
	47·0	3·85	41·0	5·34

SUMMARY

1. Coefficients of distribution for acetoin, diacetyl and acetylpropionyl between butterfat and skim-milk were measured over a range of temperatures up to 190° F. by passing preheated milk containing the reference substance through an hermetic cream separator and analysing the cream and skim-milk obtained.

2. The coefficients increased with rise in temperature, and the relationship between change in coefficient and change in temperature was linear for all three substances.

3. Coefficients of distribution for acetyl-*iso*-butyryl, acetylvaleryl, acetylcaproyl and acetylbenzoyl between butterfat and 4·5% lactose solution were measured at 40° and 60° C. by direct separation after equilibration, followed by estimation of the diketone in the lactose solution.

4. Coefficients of distribution at 100° C., obtained by linear extrapolation from these results, were: acetoin, 0·255; diacetyl, 1·27; acetylpropionyl, 5·9; acetyl-*iso*-butyryl, 20; acetylvaleryl, 23; acetylcaproyl, 152; acetylbenzoyl, 48.

5. The coefficients of distribution for diacetyl and acetylpropionyl between butterfat and 4·5% lactose solution were not affected by change in concentration of the diketone in the lactose solution.

The author is indebted to Mr B. Le Heron for assistance in the indirect measurement of the distribution coefficients at the Institute butter factory, and to Mr E. R. Elley for the analytical work.

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STEAM DISTILLATION OF TAINTS FROM CREAM

VII. BUTTERFAT/WATER DISTRIBUTION COEFFICIENTS IN RELATION TO VAPOUR/LIQUID EQUILIBRIUM COEFFICIENTS FOR TAINTING SUBSTANCES IN CREAM

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(With 6 Figures)

The concentration of a tainting substance in steam at equilibrium in the deodorization of cream is directly related to the concentration of the substance in the aqueous phase of the cream. This aqueous phase is usually a slightly diluted skim-milk. In the present series of papers the assumption is made that on the average it contains 4·5% of lactose.

The coefficients of distribution of acetoin and of some α-diketones between butterfat and 4·5% lactose solution, and of acetoin and some of the diketones between butterfat and skim-milk, have been measured over a range of temperatures (1). It has been shown that for some of them there is a marked rise in the coefficient with rise in temperature at which the equilibrium is established. The vapour/liquid equilibrium relationships for these substances in 4·5% lactose solution have also been determined (2). From the coefficients of distribution at 100° C. and the vapour/liquid equilibrium coefficients in 4·5% lactose solution, also at 100° C., the expected vapour/liquid equilibrium coefficients for cream of any specified fat content can be computed if it is assumed that steam distillation of the substance from cream is not affected by other factors, such as adsorption on the proteins. In the present paper experimental data for the vapour/liquid equilibrium relationships for the reference substances in cream are given, and the results are compared with those obtained by computation from the respective distribution ratios and the vapour/liquid equilibrium coefficients for the substances in 4·5% lactose solution.

EXPERIMENTAL

Vapour/liquid equilibrium measurements for reference substances in cream

The measurements were made in the continuous vaporization equilibrium still previously described (3,4). Estimations of diketone were made directly on the distillate, by the method of Pien, Baisse & Martin (5). The diketone was estimated in the 'deodorized' cream residue by difference, on the assumption that there was no loss of diketone during the run. The weights of distillate and residue were taken for each 4 or 5 min. run with the equilibrium still, and fat estimations were made by the Babcock method on both the original cream and the distillation residue. The calculation was then made as in the following example, representing results for a 4 min. run with the equilibrium still:

Wt. of cream residue and of distillate	= 107·3 g. and 47·5 g.
Fat content of original cream	= 38·0%
Fat content of distillation residue	= 36·75%
Concn. of reference substance in distillate (C_V)	= 9·8 μg./g.
Concn. of reference substance in original cream	= 5 μg./g.

Then

Wt. of undiluted cream taken $= \frac{36{\cdot}75}{38{\cdot}0} \times 107{\cdot}3 = 103{\cdot}8$ g.

Wt. of reference substance in cream taken $= 103{\cdot}8 \times 5 = 519{\cdot}0\,\mu$g.

Wt. of reference substance in distillate $= 47{\cdot}5 \times 9{\cdot}8 = 465{\cdot}5\,\mu$g.

Wt. of reference substance in residue $= 519{\cdot}0 - 465{\cdot}5 = 53{\cdot}5\,\mu$g.

Concn. of reference substance in residue (C_L) $= \frac{53{\cdot}5\,\mu\text{g.}}{107{\cdot}3\text{ g.}} = 0{\cdot}50\,\mu$g./g.

Hence vapour/liquid equilibrium coefficient (C_V/C_L) $= \frac{9{\cdot}8}{0{\cdot}50} = 19{\cdot}6.$

Because of the subtractions in the calculations this method, like the indirect method of measuring distribution coefficients, is subject to considerable error, but the comments made earlier (1) apply here also.

Computation of expected vapour/liquid equilibrium coefficient for cream from the corresponding coefficient for 4·5% lactose solution and the butterfat/lactose-solution distribution coefficient at 100° C.

The relation between the concentration of tainting substance in cream and the concentrations in the fat and plasma phases of the cream were computed as follows:

Let m_c = Vapour/liquid equilibrium coefficient for cream.

m_s = Vapour/liquid equilibrium coefficient for skim-milk or for 4·5% lactose solution.

C_f = Concentration of reference substance in the butterfat.

C_s = Concentration of reference substance in the skim-milk (or cream plasma), or in 4·5% lactose solution (μg./g.).

C_c = Over-all concentration of reference substance in the cream (μg./g.).

F = Fat content of cream (%).

S = Plasma content of cream (%).

k = Coefficient of distribution of reference substance between butterfat and skim-milk or between butterfat and 4·5% lactose solution at 100° C.

Then $k = C_f/C_s$, $C_f = kC_s$ and $C_s = C_f/k$,

and
$$100C_c = (SC_s) + (FC_f)$$
$$= (100 - F)C_s + FkC_s,$$
$$\frac{C_s}{C_c} = \frac{100}{100 - F(1-k)}, \qquad \text{I}$$

and
$$C_s = \frac{100C_c}{100 - F(1-k)}. \qquad \text{II}(a)$$

Or alternatively, since $F = 100 - S$,
$$C_s = \frac{100C_c}{S + k(100 - S)}. \qquad \text{II}(b)$$

Similarly,
$$C_f = \frac{100C_c}{100 - S(1 - 1/k)}, \qquad \text{III}(a)$$

or
$$C_f = \frac{100C_c}{F + (100 - F)/k}. \qquad \text{III}(b)$$

Since the vapour/liquid equilibrium coefficient of the tainting substance in cream is governed by the concentration in the cream plasma

$$\frac{m_c}{m_s}=\frac{C_V/C_c}{C_V/C_s}=\frac{C_s}{C_c},$$

i.e.

$$m_c=m_s\frac{C_s}{C_c}=m_s\frac{100}{100-F(1-k)}. \qquad \text{IV}$$

RESULTS

In Figs. 1–6 the vapour/liquid equilibrium curves for acetoin and the diketones other than diacetyl are given (curves for diacetyl in cream are shown in (10), p. 185), and these are compared with the corresponding curves for a 4·5% lactose solution. The relationships

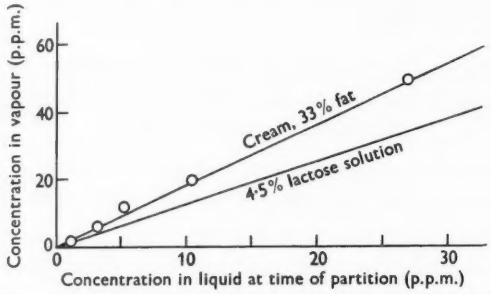

Fig. 1. Vapour/liquid equilibrium relationship for acetoin in cream of 33% fat content compared with the relationship in water or lactose solution.

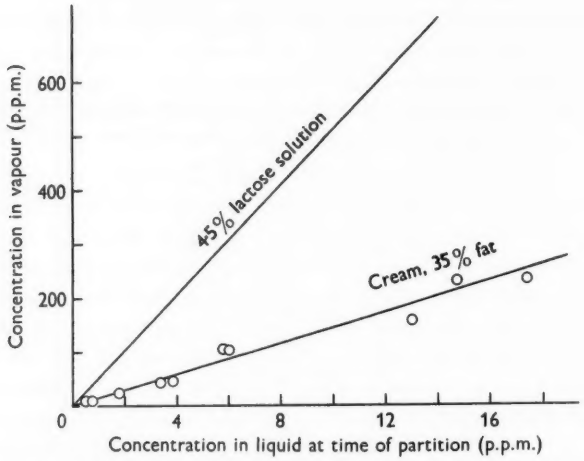

Fig. 2. Vapour/liquid equilibrium relationship for acetylpropionyl in cream of 35% fat content compared with the relationship in 4·5% lactose solution.

for all the reference substances in cream over the range of concentrations at which they were measured were thus shown to be linear. In Table 1 the vapour/liquid equilibrium coefficients represented by the linear relationships in the figures are given, along with the

corresponding coefficients for the reference substances in 4·5% lactose solutions over the range of concentrations at which these relationships also were approximately linear. The expected vapour/liquid equilibrium coefficients for the substances in creams of the

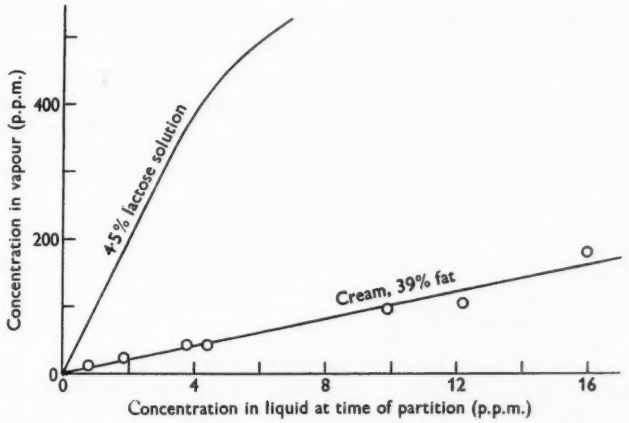

Fig. 3. Vapour/liquid equilibrium relationship for acetyl-*iso*-butyryl in cream of 39% fat content compared with the relationship in 4·5% lactose solution.

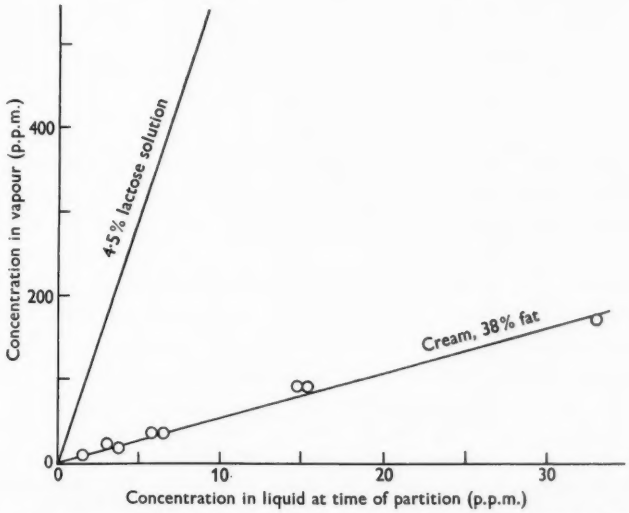

Fig. 4. Vapour/liquid equilibrium relationship for acetylvaleryl in cream of 38% fat content compared with the relationship in 4·5% lactose solution.

percentage fat contents cited at time of partition, calculated by formula IV from the equilibrium coefficients for the lactose solutions and the butterfat/lactose-solution distribution ratios at 100° C. already reported ((1), see also Table 1), are listed in Table 1.

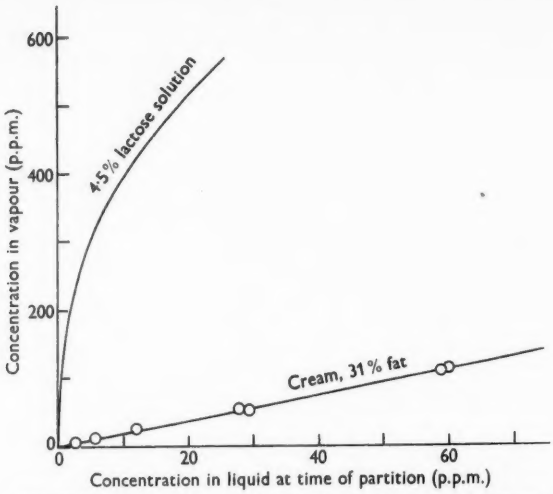

Fig. 5. Vapour/liquid equilibrium relationship for acetylcaproyl in cream of 31% fat content compared with the relationship in 4·5% lactose solution.

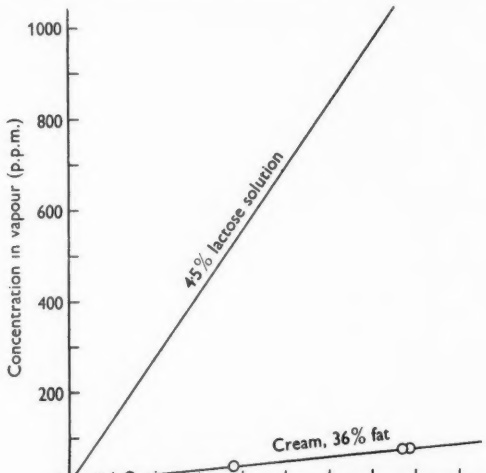

Fig. 6. Vapour/liquid equilibrium relationship for acetylbenzoyl in cream of 36% fat content compared with the relationship in 4·5% lactose solution.

DISCUSSION

Acetoin, for which the butterfat/lactose-solution distribution ratio at 100° C. was less than 1, gave a higher vapour/liquid equilibrium coefficient in cream than in 4·5% lactose solution, in agreement with the results previously reported for the constant volume distillation of acetoin from cream ((6), p. 188). For all the other reference substances, which had a butterfat/lactose-solution distribution ratio greater than 1·0, there was a

reduction in the vapour/liquid equilibrium coefficient in cream as compared with that in 4·5% lactose solution. That this reduction was due to the effect of the relative solubilities of the reference substances in butterfat and in water is shown by the reasonably close agreement between the experimental and the computed values for the vapour/liquid equilibrium coefficients in cream (last two columns of Table 1). There was no evidence that other factors, such as adsorption of the reference substance on the proteins of the cream plasma, or delay in transfer of the substance from the butterfat to the aqueous phase, had any appreciable effect on the extent of volatilization effected in the laboratory continuous vaporization equilibrium still. Since the time of contact of steam and cream in the small uptake-tube of this apparatus is only a fraction of a second (3, 4),

Table 1. *Vapour/liquid equilibrium coefficients for reference substances in* 4·5% *lactose solution and in cream, and comparison of experimental and computed coefficients for cream*

Reference substance	Fat content of cream (%)	Butterfat/lactose-solution distribution coefficient at 100° C.	Vapour/liquid equilibrium coefficient		
			Found for 4·5% lactose solution	Computed for cream	Found for cream
Acetoin	33	0·255	1·29	1·71	1·80
Diacetyl	36	1·27	30	27·3	26
Diacetyl	39	1·27	30	27·1	28
Acetylpropionyl	35	6·2	51	18·1	15·5
Acetyl-*iso*-butyryl	39	20·3	100	11·7	10·0
Acetylvaleryl	38	23·4	59	6·2	6·4
Acetylcaproyl	31	152	100	2·1	1·8
Acetylbenzoyl	36	48	13·7	0·76	0·90

it would appear that in deodorization equipment giving concurrent flow of steam and liquid there is with butterfat dispersions such as milk or cream a very rapid re-establishment of distribution equilibrium in the liquid to balance the loss of reference substance to the vapour.

From the results summarized in Table 1 it is clear that the vapour/liquid equilibrium coefficient of a tainting substance in water or aqueous lactose solution is not a reliable index of the steam volatility of the substance from milk or cream. Information is necessary also on the butterfat/water or butterfat/lactose-solution distribution coefficient. Substances readily steam volatile from water or lactose solution can be very difficult to remove from milk or cream by steam distillation. Also for optimum utilization of steam in cream deodorization the conditions of treatment may need to be varied to suit the properties of the tainting substances to be removed. In the Vacreator vacuum-pasteurizer, for example, the temperature of steam distillation can be varied over a wide range by varying the 'vacuum applied'. Other things being equal, substances for which the butterfat/lactose-solution distribution ratio increases with rise in temperature will be more easily removed at lower temperatures, and for their removal it will be advantageous to operate the equipment under a high 'vacuum applied'. Creams tainted with substances for which the butterfat/lactose-solution distribution ratio decreases with rise in temperature will correspondingly best be treated under low vacuum conditions. These and other factors will be discussed in subsequent papers in this series.

SUMMARY

1. The relationship between vapour/liquid equilibrium coefficient for a tainting substance in cream (m_c) as compared with the coefficient for the substance in skim-milk (m_s) at 100° C. is given by the formula

$$m_c = m_s \frac{100}{100 - F(1-k)},$$

where F is the percentage fat content of the cream, and k is the butterfat/skim-milk distribution coefficient at 100° C.

2. The values for the vapour/liquid equilibrium coefficients for acetoin, diacetyl, acetylpropionyl, acetyl-*iso*-butyryl, acetylvaleryl, acetylcaproyl and acetylbenzoyl in cream, computed by the formula from the equilibrium coefficients for the substances in 4·5% lactose solutions and the distribution ratios at 100° C., were in good agreement with the experimental results for these substances in cream.

3. It was shown that high solubility of a substance in butterfat relative to solubility in skim-milk greatly reduces the steam volatility of the substance from cream.

4. It was concluded that steam volatility of a substance from cream may be affected by temperature of deodorization because of the effect of temperature on the butterfat/skim-milk distribution coefficient.

Cordial thanks are due to Mr E. R. Elley for careful assistance with the analytical work, and to Dr R. M. Dolby and Mr J. K. Scott for helpful discussion.

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THE REACTION OF LACTOSE WITH ANTHRONE AND ITS APPLICATION TO THE ESTIMATION OF LACTOSE IN CASEIN AND OTHER DAIRY PRODUCTS

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(With 4 Figures)

The permanent green coloration that a solution of anthrone in concentrated sulphuric acid gives with carbohydrate material(1) is the basis of a sensitive quantitative method for the estimation of many carbohydrates. The optimum conditions for the reaction vary with the carbohydrate involved. It has been found, for example(2), that with pentoses the maximum colour is developed when the anthrone concentration is only 0·01 % (w/v) in the acid reagent, whereas with the hexoses optimum results are obtained with 0·04–0·05 % (w/v) of anthrone. The optimum time of heating also varies, that for glucose being 7·5 min.(3), whereas that for cellulose and carboxymethyl cellulose is 15 min.(4). The anthrone method has been used for determining lactose(5), but the conditions required to produce a maximum colour have not been defined. A knowledge of these optimum conditions is desirable when the method is to be applied to products such as casein which contain very little lactose. The present paper defines the optimum conditions and proposes a method for the estimation of lactose in casein.

The amount of lactose in casein provides a useful guide to the quality and conditions of manufacture and storage of the casein. The present standard methods of estimation(6,7) consist essentially of two stages. The first stage is the extraction of the lactose from the casein by shaking or soaking the casein in water for a number of hours. This procedure suffers from the disadvantage that the amount of lactose extracted by water is variable, depending on the age and history of the casein, the particle size, and the time and vigour of shaking. The method proposed in this paper overcomes this difficulty by taking the casein completely into solution as sodium caseinate and then precipitating it to leave a solution containing the lactose. The second stage is the actual determination of the lactose in the clarified extract. This is usually done by a copper reduction method which has the disadvantage that it is time-consuming, and, at the low concentrations found in most caseins, very inaccurate. The proposed method uses the anthrone reaction, which is accurate at the low concentrations found and can readily be adapted to routine analyses for the estimation of lactose.

EXPERIMENTAL AND RESULTS

Lactose solutions. A stock solution of lactose monohydrate 5 % (w/v) was kept at 0° C. From this a standard solution of lactose containing 100 μg./ml. was prepared as required.

Anthrone reagent. Anthrone (150 mg.) was dissolved in 70 % (v/v) sulphuric acid (98 %, w/w, H_2SO_4) (100 ml.). The solution was cooled and aged for at least 2 hr. (preferably overnight) at 0° C. before use.

Glassware. All glassware was cleaned in a detergent solution, then well rinsed with warm tap water and finally with distilled water before drying. Care was taken to ensure that tubes and stoppers were free from cellulose fragments from cloths.

Proposed method for the determination of lactose in casein and other dairy products

Preparation of solution for analysis

Casein. Casein (1 g.) and sodium hydrogen carbonate (0·1 g.) are placed in a large boiling tube, and water (25 ml.) is added. The mixture is warmed with stirring in a bath at 60°–70° C. until the casein is dissolved (10 to 15 min.). 0·1 N-sulphuric acid (25 ml.) is then added while the solution is stirred. The precipitated casein is removed by filtration and the filtrate retained for lactose estimation.

Milk. Milk (1 ml.) is pipetted into a volumetric flask and diluted to 500 ml. with water.

Estimation of lactose with the anthrone reagent. Aliquots (1 ml.) of the solutions to be analysed containing 0–100 μg. of lactose are pipetted into boiling tubes which are then placed in an ice bath and shaken while ice-cold anthrone reagent (10 ml.) is added. The tubes are stoppered, and the contents well mixed. They are then placed in a boiling water-bath for 6 min. and then transferred to the ice bath for 30 min. The optical densities of the coloured solutions are then read at 625 mμ. A blank consisting of water (1 ml.) and anthrone reagent, and a standard containing 100 μg./ml. of lactose and anthrone reagent are included in each batch of analyses. To allow for slight variations between different sets of results due to instability of anthrone in sulphuric acid, results are calculated by reference to the standard included in the batch, and not by reference to a previously determined plot of optical density against concentration of lactose such as is shown in Fig. 1.

Establishment of optimum conditions of reaction

Heat of hydration of anthrone reagent. A possible source of error in the anthrone method is the production of colour due to a rise in temperature when the anthrone reagent is added to the sugar solution. This difficulty has been partially overcome by preparing the anthrone reagent in 70% (v/v) sulphuric acid rather than in the 90% (v/v) acid used by Fagen *et al.*(5), so that the heat of hydration has been largely dissipated before the reagent is added to the lactose solution. If then both the lactose solution and the anthrone reagent are cooled to near 0° C. before mixing the source of error is eliminated.

Effect of anthrone concentration on the reaction. Samples of anthrone reagent (10 ml.) containing anthrone (0·005 to 0·3% (w/v)) in 70% (v/v) sulphuric acid were heated with standard lactose solution (1 ml.) for 6 min. The optical density of each solution was read against a blank of the same anthrone solution and water (1 ml.). The results are shown in Fig. 2 as a plot of optical density at 625 mμ. against anthrone concentration of the reagent. It can be seen that optimum results were obtained with 0·15% (w/v) anthrone reagent.

Effect of heating time on the reaction. Samples (1 ml.) of lactose solution (100 μg./ml.) were heated with the anthrone reagent in a boiling water-bath. At intervals samples were transferred to an ice bath. The results are shown in Fig. 3 as a plot of optical density at 625 mμ. against time in the boiling bath. It can be seen that the colour reached a maximum with a heating time of 6 min.

Absorption spectrum of developed colour. The absorption spectrum of the colour produced by lactose (100 μg./ml.) with the anthrone reagent is shown in Fig. 4 measured in a 1 cm.

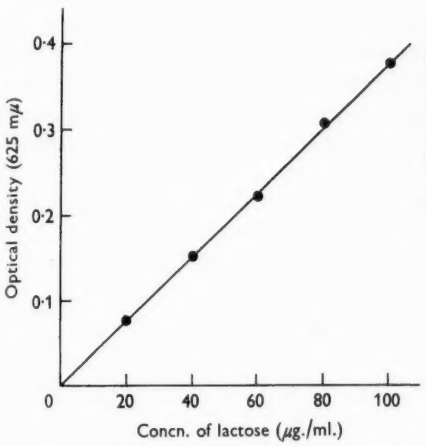

Fig. 1

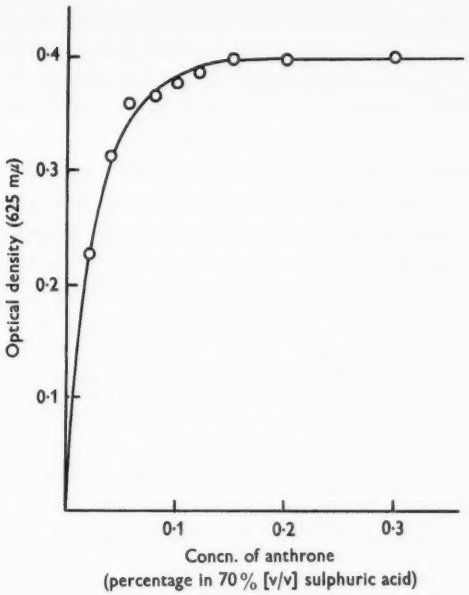

Fig. 2

Fig. 1. Relationship of lactose concentration to optical density after reaction with anthrone reagent (0·15% anthrone in 70% (v/v) sulphuric acid) for 6 min. at 100° C.

Fig. 2. Effect of anthrone concentration on the optical density of the coloured solution produced from 100 μg. lactose after 6 min. at 100° C.

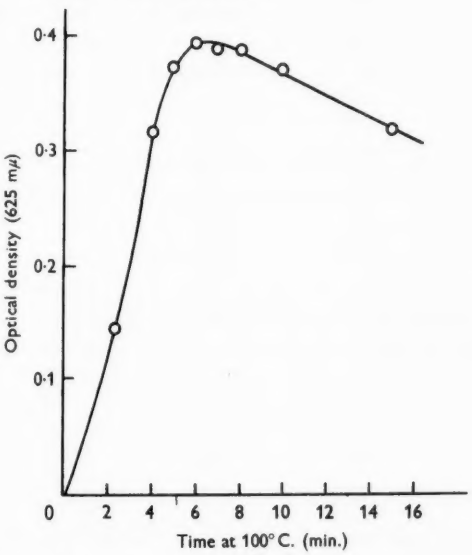

Fig. 3

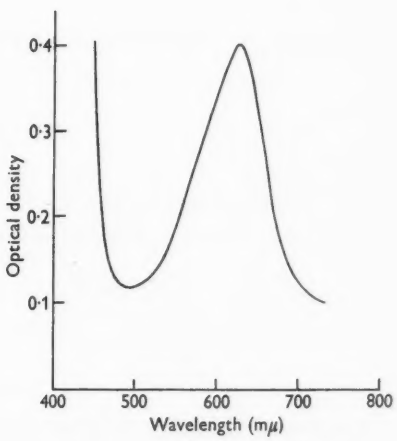

Fig. 4

Fig. 3. Effect of heating time on the optical density of the coloured solution produced from 100 μg. of lactose with anthrone reagent (0·15% anthrone in 70% (v/v) sulphuric acid) for 6 min. at 100° C.

Fig. 4. Absorption spectrum of the coloured solution produced by 100 μg. of lactose after heating with anthrone (0·15% in 70% (v/v) sulphuric acid) for 6 min. at 100° C.

corex cell. The absorption maximum at 625 mμ. which lactose gives with the reagent is at the same wavelength as that given by other carbohydrates (2, 3, 4, 8).

Recovery of lactose added to casein. Casein (5 g.) and sodium hydrogen carbonate (0·5 g.) were dissolved in water (125 ml.) at 60° C. Portions (25 ml.) of the solution were taken and 0, 2, 5 and 10 ml. of 0·05 % lactose solution were added to them, followed by 25, 23, 20 and 15 ml. respectively of 0·1 N-sulphuric acid to precipitate casein while keeping the volume constant. The solutions were well mixed and filtered, and the lactose was determined on the filtrates. The recovery of added lactose was calculated by deducting the amount of lactose in the blank. The results given in Table 1 are the average of many determinations, and they show that with the proposed method lactose recovery is almost complete.

Table 1. *Recovery experiments with the proposed anthrone method of lactose added to casein solution*

Added lactose (μg./ml.)	Average recovered (μg./ml.)	Extreme variations (μg./ml.)
0	0	0
20	19·9	±2·0
50	50·7	±1·5
100	98·7	±0·5

Table 2. *Comparison of British Standard method and the proposed anthrone method for the estimation of lactose in casein*

	Percentage lactose	
Sample	B.S. method	Proposed method
Australian casein	0·037	0·35
	1·0	1·3
	0·4	0·85
	0·77	1·1
	0·88	1·2
New Zealand lactic casein	0·11	0·13
	Nil	0·095
	Nil	0·06
	Nil	0·05
	Nil	0·08
	0·037	0·095
	Nil	0·07

Table 3. *Comparison of copper reduction method and the anthrone method for the estimation of lactose in milk*

Sample	Copper reduction	Anthrone
Homogenized milk	4·72	4·75
Milk 1	4·45	4·52
Milk 2	4·52	4·58
Skim-milk	4·81	4·92

Comparison of British Standard method (6) and the proposed anthrone method for the estimation of lactose in casein. The lactose content of a number of casein samples was determined by both the British Standard method and by the proposed anthrone method. The results, tabulated in Table 2, show that in every case less lactose was found in the casein by the British Standard method than by the proposed method.

Comparison of the copper reduction method and the anthrone method for the estimation of lactose in milk. The results of the comparison of a number of samples are shown in Table 3. The anthrone method gave results which were on the average 0·067% higher than the copper reduction method.

DISCUSSION

The mechanism of the reaction of anthrone with lactose is almost certainly a rapid hydrolysis of the disaccharide to glucose and galactose, followed by conversion of these hexoses to 5-hydroxymethylfurfural which reacts with the anthrone (8). It would therefore be expected that the optimum conditions for the reaction would be similar to those found for the hexoses, and this proved to be correct.

The high recovery of lactose added to casein shows that the proposed method for the estimation of lactose in casein is quantitative. The results obtained by this method were in all cases higher than those given by the British Standard method with the same caseins, which emphasizes that in the standard method extraction of lactose is not complete. The results with milk confirm those of Fagen *et al.* (5) and are in agreement with his statement that the anthrone method is a rapid and sensitive colorimetric method for the determination of lactose in milk and many other dairy products.

SUMMARY

Lactose was estimated accurately and rapidly in casein by use of anthrone reagent on the clear filtrate from precipitation of a sodium bicarbonate solution of the casein with 0·1 N-sulphuric acid. The reagent consists of 0·15% (w/v) anthrone in 70% (v/v) sulphuric acid. The ice-cold anthrone solution (10 ml.) was added, with cooling, to 1 ml. of the ice-cold solution containing 0–100 μg. of lactose. The mixture was heated at 100° C. for 6 min. and cooled for 30 min. The optical density of the coloured solution was then measured at 625 mμ.

The author wishes to thank Dr F. H. McDowall for helpful advice and criticism and Miss M. A. Cowling for assistance with the experimental work.

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PHYSICAL CHANGES IN MILK CAUSED BY THE ACTION OF RENNET

IV. EFFECTS OF VARYING RENNET CONCENTRATION AND TEMPERATURE

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(With 1 Figure)

In two earlier papers in this series (which will be referred to as I and III) Scott Blair & Burnett(1, 2) described a method for measuring the increasing rigidity of rennetted milk and the effects of various milk treatments on the rigidity moduli and viscosities. The following nomenclature is used: over-all modulus G, 'immediate' modulus G_1, 'slow' modulus G_2, external creep viscosity η_1 and internal damping viscosity η_2. All these parameters give good straight lines during most of the setting time (t) when plotted against $\log t$. Their slopes ($dG/d(\log t)$ or $\dot{G}t$, etc.) and their intercepts on extrapolation to the time axis (I), give convenient measures, respectively, of the (logarithmic) rates of setting of the gels and of what may be called the 'induction periods', slightly longer than the observed coagulation times.

Very good reproducibility can be got, using a suitable reconstituted fat-free dried milk with carefully controlled rennet concentration, temperature, amounts of added calcium chloride, lactic acid, etc. In this series of papers, each in turn of these conditions is varied, the remainder being held constant. The present paper is concerned with variations, first, in the concentration of rennet and secondly in the temperature.

EXPERIMENTAL

Experiment 1. *Effects of variations in rennet concentration*

With all other factors kept constant, samples of the dried milk used as standard were subjected to various concentrations of rennet, and the over-all modulus G was determined during subsequent setting at 32° C. The results are shown in Table 1.

Table 1. *Effect of variations in rennet concentration* (C) *on logarithmic slopes* ($\dot{G}t$) *and intercepts* (I) *of setting curd*

(t_c is the observed 'coagulation'; $\dot{G}t$ is defined as the increase in G when t is doubled.)

C (parts per 10^4)	$\dot{G}t$ (dynes/cm.^{2})	I (min.)	t_c (min.)	$C \times (\dot{G}t)^3 \times 10^{-7}$	$C \times I$	$C \times t_c$
10	147	4·0	2·75*	3·17	40	28
7	150	4·7	3·0	2·36	33	21
4	175	8·0	5·0	2·14	32	20
2	235	13·3	9·0	2·59	27	18
1·5	235	16·0	11·25	1·95	24	17
1·0	295	25·0	18·0	2·54	25	18
0·75	320	34·5	25·0	2·46	26	19
0·5	375	52·5	40·0	2·64	25	20

* This reading lacked precision.

It is seen from the table that $\dot{G}t$ decreases progressively with increasing concentration of rennet and that, except at the highest concentration, it varies inversely as the cube root of the concentration. Both I and t_c decrease with increasing concentration to which they are approximately inversely proportional. It should again be emphasized that $\dot{G}t$ is a *logarithmic* rate of increase. It is most convenient to use this, because it is a constant, independent of setting time. But its decreasing with increasing concentration of rennet does not imply that the rate at any given time also decreases. Such rates, however, would have little value since they depend on the arbitrary choice of a time.

Experiment 2. *Effect of varying temperature in normal setting process*

Using the method described in paper III, the Burgers parameters were separately determined in this experiment, as well as λ, the proportion of elastic recovery which is 'slow', $t_{\text{ret.}}$, the retardation time, $t_{\text{rel.}}$, an indirectly determined relaxation time (calculated from η_1/G_1) and the ratio $t_{\text{rel.}}/t_{\text{ret.}} = N$, called the 'springiness number'. Experiments were also done at each temperature subjecting the curd to a long continuous stressing in one direction, the 'creep' after 100 min. being measured, after which the curd was allowed to recover. In these creep experiments, considerable amounts of whey were pushed through the curd so that the concentration was changing. In spite of this, such experiments have considerable theoretical interest and will be described elsewhere. For classification purposes, two figures are here given: ϵ_{100} is the over-all creep in cm. as recorded just before release (i.e. about 100 times the actual displacement) and ϵ'_{20} is the recorded elastic recovery 20 sec. after release. Since the interest here lies in the creep, i.e. the slow movements of the curd, the 'quick' elastic displacements taken after 5 sec. loading and again after 5 sec. recovery, were subtracted from the total readings. The data for ϵ_{100} and ϵ'_{20} in Table 2 were corrected in this way.

Table 2. *Effect of temperature changes on rheological parameters*

(*Note.* The same (best) value of I is taken for all the curves of Burgers parameters.)

Temp. (° C.) ...		21	25	29	32	35	38	41
min.	t_c	35?	16	12	10	7	6·5	6·25
	I	35	26	18	15	9	9	7·5
dynes/cm.^{2}	$\dot{G}t$	70	137	165	182	157	155	120
	$\dot{G}_1 t$	98	223	258	330	290	265	200
	$\dot{G}_2 t$	253	345	448	430	340	360	290
poises $\times 10^{-3}$	$\dot{\eta}_1 t$	16·7	21·3	28·8	25·5	22·0	26·0	18·0
	$\dot{\eta}_2 t$	10·0	11·3	14·7	14·0	10·0	10·0	9·0
	λ	0·28	0·34	0·35	0·43	0·45	0·40	0·40
sec.	$t_{\text{ret.}}$	41·6	35·1	35·9	32·9	31·6	30·0	30·5
	$t_{\text{rel.}}$	169	99·7	95·9	76·8	74·1	81·5	94·0
	N	4·05	2·97	2·73	2·32	2·32	2·78	3·06
Recorder readings (cm.)	ϵ_{100}	24·4	28·4	35·9	44·1	49·8	83·4	79·5
	ϵ'_{20}	7·9	5·9	7·9	8·95	9·4	11·5	12·8

It will be noticed that some of the parameters (t_c, I, $t_{\text{ret.}}$, ϵ_{100} and ϵ'_{20}) are single-valued functions of temperature. The first three fall progressively and the last two rise as temperature is increased. All the elastic constants ($\dot{G}t$, $\dot{G}_1 t$ and $\dot{G}_2 t$), $t_{\text{rel.}}$, N, and (probably) λ and $\dot{\eta}_1 t$, show maximal or minimal values at somewhere about the normal working temperature of the enzyme. The internal viscosity does not seem to change its rate of development ($\dot{\eta}_2 t$) much within the range of temperature studied; but this is one of the

most difficult parameters to measure accurately. It may possibly be passing through a maximum. The parameters, which are single-valued functions of temperature, give fairly good straight lines when their logarithms are plotted against temperature (θ° C.). The most accurately determined is I, the plot of which is shown in Fig. 1. This plot corresponds to the simple equation $I = e^{B-A\theta}$.

Relaxation experiments were also carried out at all these temperatures. A pressure was applied to the curd for 5 sec., at the end of which time the displacement was noted and, from then on, held constant for 5 min. by systematically reducing the pressure which was recorded during the stress relaxation. The results, for a temperature of 32° C., have much theoretical interest and are discussed elsewhere(3), but the relationship between relaxation and temperature could not be clarified without very much further work.

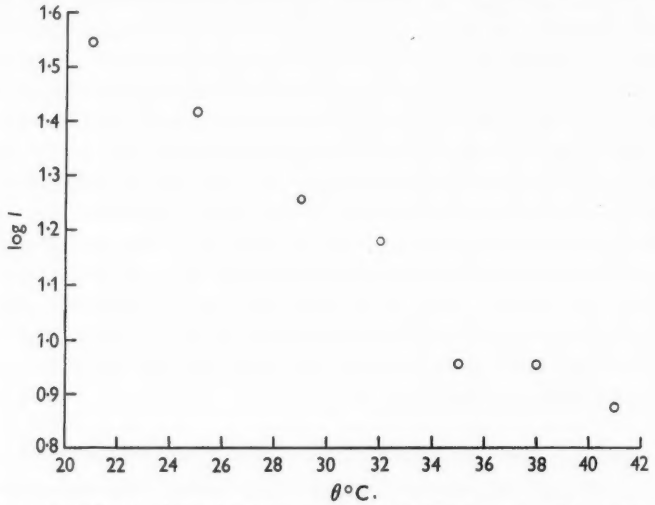

Fig. 1. Logarithmic relationship between intercept of G/log t curves and temperature.

The reason for this is the following. Both creep and relaxation experiments should be done, at each temperature, at such an age of the curd as would give approximately identical values of G; i.e. the gels should be equally firm. This is done by trial and error and, in the case of the creep experiments, the inevitable differences in G were not serious enough to matter. In the relaxation experiments, however, it became clear that the results were much too dependent on chance variations of G and quantitative conclusions about the effects of temperature could therefore not be drawn.

It has been found, however(4), that at about 32° C. the relaxation times in the curd are distributed in a comparatively simple way which probably corresponds to a normal (Gaussian) distribution of entropies, whereas at appreciably higher or lower temperatures, a much more complex distribution appears. The significance of this finding is not yet understood.

DISCUSSION

It is somewhat difficult to compare these results with data published by other workers since the contributions of the immediate and slow elastic reactions and of the external and internal viscosities have not been independently determined by other investigators.

Hansson, Sjöström & Samuelsson (5) measured changes in 'elasticity' during the setting of milk, but they themselves point out (quotations from English summary) that 'this expression is not quite correct...' since the property measured 'depends not only on the elasticity but also on the plastic deformations of the curd'. Nevertheless, some comparison with their results is of interest. Their finding that the relation between 'elasticity' and rennet concentration is linear except at high concentrations tallies with the linear relation between concentration and intercept in Table 1 above, but not with the cube-root relation with $\dot{G}t$. It must be remembered, however, that $\dot{G}t$ is a measure of *logarithmic* rate of setting. Experiments on the variation of the individual Burgers parameters with changes in rennet concentration do not show anything very different from the data already given in Table 1.

With regard to temperature, Hansson *et al.* give some rather complex curves (their fig. 6), each containing only four points (i.e. four readings during setting) for temperatures at 5° intervals from 25° to 45° C. These curves cross one another and are difficult to interpret, but the authors themselves conclude that 'the greatest elasticity was observed at 35° C.'. In Table 2 above, the maxima in the values of $\dot{G}t$, etc., occur at 32° C. The maximum for λ is at 35° C. The springiness number appears to be lowest and the creep viscosity increase ($\dot{\eta}_1 t$) highest at 29° C. In spite of differences in experimental conditions, it seems that many parameters do inflect at about 30°–35° C.

Berridge (6, 7) has discussed the double nature of rennet clotting, and reports experiments in which rennet was allowed to complete the first stage of its action at 0° C., clotting afterwards taking place at a series of temperatures. The logarithm of the clotting time was found to be linearly related to temperature.

The authors have done a number of preliminary experiments in which different quantities of rennet were allowed to act on milk for different times at temperatures of 1°–2° C., and these give very complex results. Experiments are also in progress in which rennet concentration and time in the cold are fixed and the temperature of the milk is then quickly raised to different levels. It will be interesting to see whether the values of $\dot{G}t$ and I so obtained are exponentially related to temperature. Already it may be said, however, that the same function related $\log I$ and θ in Fig. 1 (the setting having been done in the normal way) as that which related $\log t_c$ to θ in Berridge's measurements, made during the second phase of coagulation only.

SUMMARY

1. Under carefully standardized conditions, the increase in the over-all rigidity modulus of curd when the time after rennetting is doubled, varies inversely as the cube root of the rennet concentration. The intercepts of the extrapolated setting curves on the time axis and the observed coagulation times vary inversely as the concentration.

2. With the same (normal) rennet concentration, some rheological parameters increase or decrease progressively as temperature is increased from 21° to 41° C.; others pass through maxima or minima, all between 29° and 35° C.

3. Long-term 'creep' experiments have been done over the above temperature range, creep and slow recovery increasing progressively with temperature. Relaxation experiments, though showing the simplest distribution of relaxation times at about 32° C.,

are too greatly influenced by unavoidable differences in firmness at the different temperatures for it to be possible, at this stage, to draw conclusions about their temperature dependence.

4. A comparison of some of these results with data published by a few other workers is attempted.

We are indebted to Mr A. Graham and to Mr C. S. Landau for helpful discussions especially concerning the 'creep' experiments, which were done with their collaboration.

We would also thank Dr N. J. Berridge for reading the manuscript of this paper and checking the calculations.

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FURTHER STUDIES ON UNRESTRICTED WHEY FOR FATTENING PIGS INCLUDING THE EFFECT OF OMITTING ANTIBIOTIC FROM THE DIET DURING THE LATER STAGES OF FATTENING

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(With 1 Figure)

In an earlier trial on feeding unrestricted amounts of whey, carried out in the Danish piggery at East Farm, of Cow and Gate Farms Ltd., Dorset (1), it was shown that where the aim was to utilize as much whey and as little meal as possible, a reasonably satisfactory performance could be obtained by feeding 3 lb. of basal meal, reduced to 2 lb./pig/day by 13 weeks of age, plus unrestricted amounts of whey. However, the comparison of the all-meal fed control pigs and the whey-fed animals was influenced by the relatively unsatisfactory performance of the former. In addition, in more recent experiments carried out on the Cow and Gate farm, a considerable improvement in the over-all performance of all pigs as compared with the earlier trials has been observed (2). It was felt, therefore, that it would be useful to carry out a further similar trial comparing the feeding of a diet of meal only with a diet of meal plus unrestricted amounts of whey.

In all the previous pig fattening experiments completed on the Cow and Gate farm (1, 3, 4, 2) an antibiotic was included in the diet of all pigs during the entire fattening period. Many experiments have been reported on the effect of omitting the antibiotic from the diet of meal-fed pigs in the later stages of the fattening period. In some of these, better results were obtained by feeding the antibiotic throughout the fattening period, whereas in others there was no apparent difference between pigs fed antibiotics for the entire fattening period or for only the first part of it (see review by Lucas (5)). An investigation was included in the present experiment, therefore, on the effect under the prevailing conditions at the Cow and Gate farm, of omitting the antibiotic from the diet during the latter part of the fattening period.

EXPERIMENTAL

Treatments. There were four treatments:

(i) Meal only fed *ad lib.* up to a daily maximum of 6 lb./pig with antibiotic included for the whole of the fattening period.

(ii) As (i) but antibiotic withdrawn at an average pen live weight of 130 lb.

(iii) Three lb. meal reduced to 2 lb./pig/day by 13 weeks of age plus unrestricted whey, with antibiotic included for the whole fattening period.

(iv) As (iii) but antibiotic withdrawn at an average pen live weight of 130 lb.

Pigs. Details of the pigs used, their pre-experimental management, breeds, selection for experiment and housing were all identical with those described in detail by Braude *et al.* (1) with the exception that the additional breeds and crosses listed by Braude *et al.* (4) were again included. New concrete beds having a greater fall than previously were fitted in all pens before the experiment started. This was subsequently found to maintain the sleeping quarters of the pigs in a much drier condition than had been possible before. There were five pens of nine pigs on each of the four treatments, making a total of 180 pigs for the experiment. The four treatments were randomly allocated to the pens within each of the five replicates. The first two replicates started on 26 April, the next two on 3 May and the fifth on 17 May 1956. The average age of the pigs at the start of a replicate was approximately 8 weeks.

Table 1. *Percentage composition of the meal mixtures used on the four experimental treatments*

	Meal mixture no.				
	1	2	3	4	5
	Fed to pigs on treatments				
	(i) and (ii) up to 130 lb. live weight	(i) from 130 lb. to slaughter	(ii) from 130 lb. to slaughter	(iii) From weaning to slaughter; (iv) up to 130 lb. live weight	(iv) From 130 lb. to slaughter
Barley meal	55	63	63	55	55
Fine millers offal	35	30	30	35	35
White fish meal	10	7	7	10	10
Terramycin supplement* (lb./ton)	2	2	—	3	—
Vitamealo 'Supercon' supplement (lb./ton)†	2½	1¼	1¼	3¼	3¼

* Pfizer Ltd. containing 5 g. oxytetracycline/lb.

† Agricultural Food Products Ltd., containing in 5 lb.: 6 million i.u. vitamin A; 1½ million i.u. vitamin D_3; Riboflavin, nicotinic acid and trace minerals including zinc.

Experimental feeding of pigs. During the first fortnight after being put into the experimental pens, the pigs were fed the same creep pellets, containing penicillin, that they had received from 3 weeks of age. During the third week the pellets were gradually replaced by the various experimental meal mixtures as given in Table 1. The antibiotic used was oxytetracycline. Since meal mixtures 4 and 5, fed to the pigs receiving whey, were restricted to a maximum of 2 lb./pig/day from 13 weeks of age, higher levels of the antibiotic and the vitamin and mineral supplements were included in these two mixtures, than in mixtures 1, 2 and 3 given to the all-meal fed animals.

During the first week after being put into the experimental pens, all pigs continued to receive the unrestricted supply of whey which they had had since birth. At the end of the first week, the whey supply for the pigs on treatments (i) and (ii) was abruptly stopped, and water was made available.

The pigs were fed once daily in the afternoon.

Records. The pigs were individually weighed in the morning once weekly for the first three weeks of the experiment and then fortnightly: as the animals approached slaughter weight, they were weighed twice weekly. All food (meal and whey) given was recorded.

The whey was delivered to 40-gallon individual pen tanks through a volume meter and recorded in gallons. The pen tanks were connected to an automatic drinking trough in each pen. On the same day as the pigs were weighed, all refusals of meal and whey were determined, so that records of total food consumption by each pen of pigs were obtained fortnightly throughout the experiment. The method used to adjust the food consumption data to allow for pigs that either died or had to be taken off the experiment and the method of conversion of whey consumed to a 12% moisture basis (used in arriving at the over-all figure of efficiency of food conversion for the whey-fed pigs) were as described by Braude *et al.* (1).

Carcass grading. On the first occasion that a pig exceeded 200 lb. live weight at the twice weekly weighings, it was taken out of the experimental pen and sent to slaughter the following morning without receiving further food. All pigs were sent to the same local bacon factory. Other details of the slaughter arrangements and carcass measurements taken were as described by Braude *et al.* (1).

Health of pigs. During the course of the experiment, five out of the total of 180 pigs, or under 3%, either died or had to be taken off experiment, the reasons for their removal being given in Table 2.

Table 2. *Details of pigs which died or were taken off experiment*

Treatment no.	No. of pigs involved	Cause of death or removal from experiment
(i)	1	Sudden loss of condition, cause unknown
(ii)	1	Died, no obvious cause found on P.M.
(iii)	1	Died, cause unknown
(iv)	1	Died, acute pneumonia
	1	Rupture

As in all previous experiments carried out at East Farm, symptoms of virus pneumonia were observed in the pigs. The same routine injections to control the secondary complications of this condition as previously used (1) were again given and a reasonably satisfactory over-all rate of growth was maintained by most of the pigs. The incidence of secondary complications was rather less than in some of the previous trials, the total number of pigs receiving at least one course of injections being 5, 2, 8 and 10 on treatments (i), (ii), (iii) and (iv) respectively, that is, 7 out of 90 pigs on all-meal feeding and 18 out of 90 pigs on whey feeding.

All the experimental animals were sprayed with benzene hexachloride shortly after they entered the fattening house, as a precaution against sarcoptic mange. Suspected cases of mange occurred later in 3 pigs. These were scrubbed with benzene hexachloride which satisfactorily cleared up the trouble, the affected animals suffering no apparent set-back in growth.

RESULTS

The mean results for live-weight gain, efficiency of food conversion and carcass measurements, together with appropriate standard errors are given in Table 3. The standard errors were calculated from randomized block analyses of variance on the pen means, no adjustments being made for variation in either initial live weight or cold dead weight (6). The experiment was designed as a 2×2 factorial and the results of the appropriate analysis are given in Table 4 with the standard errors and statistical significance of the treatment

Table 3. *Treatment means for live-weight gain, efficiency of food conversion and carcass measurements of pigs on experiment from approximately 8 weeks of age to a mean live-weight of 202 lb.*

	Treatment no.				
	All meal		Meal + whey		
Period when antibiotic fed ...	(i) To slaughter	(ii) To 130 lb. live weight	(iii) To slaughter	(iv) To 130 lb. live weight	Standard error of means*
No. of pigs	44	44	44	43	—
Days on experiment	114·7	114·8	132·9	135·4	—
Daily gain (lb.)	1·34	1·34	1·15	1·13	0·020
Efficiency of food utilization (lb. meal or meal plus 88% dry matter whey/lb. gain)	3·74	3·69	3·80	3·85	0·046
Dressing percentage	76·0	76·0	74·8	75·5	0·26
Carcass length (mm.)	780·9	781·7	791·6	785·5	2·77
Shoulder fat (mm.)	51·3	50·8	49·2	49·0	0·48
Mid-back fat (mm.)	27·7	27·1	26·7	27·3	0·44
Loin fat (mm.) 1	35·6	35·1	33·4	33·2	0·57
2	32·5	31·7	29·6	29·6	0·52
3	40·0	40·0	37·8	37·7	0·84
Belly thickness (mm.) (average of 3 points)	35·7	35·9	36·1	36·0	0·24
Eye muscle (mm.) Depth	38·9	39·7	39·9	39·9	0·32
Breadth	80·2	81·2	80·9	80·9	0·58

* Based on 12 degrees of freedom.

Table 4. *Treatment constants and their standard errors, and the statistical significance of the treatment effects and interactions*

	Basic ration			Antibiotic included to				
	Meal (1)	Meal plus whey (2)	Significance of treatment mean square† (3)	Slaughter (4)	130 lb. live weight (5)	Significance of treatment mean square† (6)	Standard error of means (7)	Significance of interaction (8)
Daily gain (lb.)	1·34	1·14	***	1·25	1·23	N.S.	0·014	N.S.
Efficiency of food utilization								
(i) lb. meal/lb. gain	—	1·90	—	—	—	—	—	—
(ii) gal. whey/lb. gain	—	2·65	—	—	—	—	—	—
(iii) lb. meal, or meal plus 88% dry matter whey/lb. gain	3·72	3·83	*	3·77	3·77	N.S.	0·033	N.S.
Dressing percentage	76·0	75·1	**	75·4	75·7	N.S.	0·18	N.S.
Carcass length (mm.)	781·3	788·6	*	786·2	783·6	N.S.	1·96	N.S.
Shoulder fat (mm.)	51·1	49·1	**	50·2	49·9	N.S.	0·34	N.S.
Mid-back fat (mm.)	27·4	27·0	N.S.	27·2	27·2	N.S.	0·31	N.S.
Loin fat (mm.) 1	35·4	33·3	**	34·5	34·2	N.S.	0·40	N.S.
2	32·1	29·6	***	31·0	30·6	N.S.	0·37	N.S.
3	40·0	37·8	*	38·9	38·9	N.S.	0·59	N.S.
Belly thickness (mm.) (average of 3 points)	35·8	36·0	N.S.	35·9	35·9	N.S.	0·17	N.S.
Eye muscle (mm.)								
Depth	39·3	39·9	N.S.	39·4	39·8	N.S.	0·23	N.S.
Breadth	80·7	80·9	N.S.	80·6	81·0	N.S.	0·41	N.S.

Col. 1. Mean of treatments (i) and (ii). Col. 2. Mean of treatments (iii) and (iv). Col. 3. Significance of difference between cols. 1 and 2. Col. 4. Mean of treatments (i) and (iii). Col. 5. Mean of treatments (ii) and (iv). Col. 6. Significance of difference between cols. 4 and 5. Col. 7. Standard error of each of means in cols. 1, 2, 4 and 5. Col. 8. Significance of interaction between the effects of the different stages to which antibiotic was fed and the nature of the basic ration.

† Significance levels: N.S., $P > 0{\cdot}05$; *, $0{\cdot}05 > P > 0{\cdot}01$; **, $0{\cdot}01 > P > 0{\cdot}001$; ***, $P < 0{\cdot}001$.

effects and interactions. The commercial grading results are summarized in Table 5, and in Table 6 the pattern of food consumption over 7-day periods for the first 3 weeks of the experiment and subsequently over 14-day periods is shown. In Fig. 1 the average growth curves of the pigs fed an all-meal diet and of those fed meal plus whey are given from 8 weeks of age up to the time the first pigs went away to slaughter.

Table 5. *Commercial grading* results of pigs*

Treatment	No. of pigs	Final grade: Triple A No.	Triple A %	A No.	A %	B No.	B %	C No.	C %	% grade A at: Mid-back	Loin	Shoulder
(i)	44	16	36·4	22	50·0	16	36·4	6	13·6	75·0	38·6	63·6
(ii)	44	15	34·1	31	70·5	10	22·7	3	6·8	86·4	43·2	72·8
(iii)	44	23	52·3	35	79·5	8	18·2	1	2·3	90·9	59·1	81·8
(iv)	43†	22	52·4	33	78·6	7	16·7	2	4·7	92·9	57·1	78·6
All meal	88	31	35·2	53	60·2	26	29·6	9	10·2	80·7	40·9	68·2
Meal plus whey	86	45	52·3	68	79·1	15	17·4	3	3·5	91·9	58·1	80·2

* As in force at time pigs were slaughtered.
† Includes one rig pig not graded.

Table 6. *Mean consumption of meal and whey/pig/day over 7-day periods for first 3 weeks of the experiment, and subsequently over 14-day periods up to the time when the first pigs went to slaughter*

Approximate age of pigs (weeks)	All meal (88 pigs). Meal (lb.)	Meal + whey (87 pigs): Meal (lb.)	Whey (gal.)	Meal + 88% dry matter whey (lb.)
8–9	2·1	2·1	0·69	2·6
9–10	3·1	2·8	0·96	3·5
10–11	3·4	2·7	0·98	3·4
11–13	3·9	2·7	1·30	3·6
13–15	4·7	2·0	2·28	3·7
15–17	5·2	2·0	3·03	4·2
17–19	5·7	2·0	3·51	4·6
19–21	5·9	2·0	3·78	4·8
21–23	6·0	2·0	4·16	5·0
23–25	6·0	2·0	4·44	5·2

An average daily peak consumption of 4·51 gal. of whey per pig was reached during the 25th to 27th week of age.

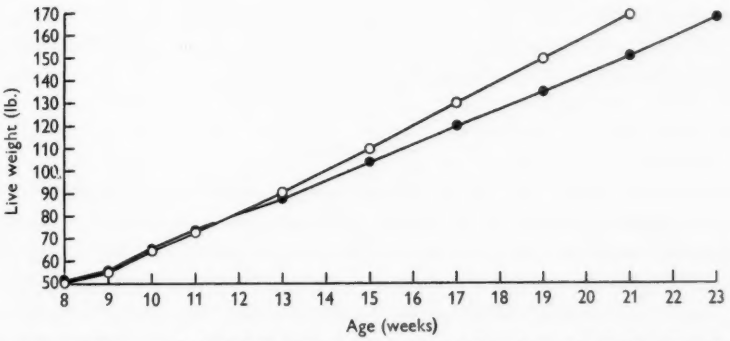

Fig. 1. Average growth curves of pigs fed all meal, ○—○, and meal plus whey, ●—●, from 8 weeks of age to time when first pigs were sent to slaughter.

Comparison of all-meal feeding with meal plus unrestricted amounts of whey

(*a*) *Live-weight gain and efficiency of food utilization.* The rate of growth and efficiency of food utilization of the pigs receiving the all-meal diet were significantly better than for the pigs given the basal meal plus unrestricted access to whey.

(*b*) *Dressing percentage.* The all-meal fed pigs had a significantly higher dressing percentage (cold carcass weight as a percentage of the unstarved live weight on the day before slaughter) than the pigs receiving whey.

(*c*) *Carcass length.* The whey-fed pigs had significantly longer carcasses than the all-meal fed animals.

(*d*) *Back fat.* The whey-fed pigs had significantly thinner shoulder and loin fat measurements than the pigs fed the all-meal diets. The difference in the mid-back fat thickness between the two groups was not significant.

(*e*) *Average belly thickness, depth and breadth of eye-muscle.* The differences in these three measurements between the all-meal fed and whey-fed pigs were all very small and none was significant.

(*f*) *Commercial grading results.* The significantly thinner fat measurements along the back of the whey-fed pigs were reflected in their over-all superior commercial grading, as shown in Table 5, compared with the all-meal fed animals.

Effect of omission of antibiotic at an average pen live weight of 130 *lb.*

The results in Tables 3 and 4 show that removing the antibiotic from the diet, whether all meal or meal plus unrestricted amounts of whey, had no significant effect on growth rate, efficiency of food utilization, dressing percentage or any of the carcass measurements. Similarly, there were no marked differences in the commercial grading results given in Table 5, arising from omission of the antibiotic from the diet from 130 lb. live weight.

Interactions

There were no significant interactions between the effects of type of diet and stage to which antibiotic was fed for any of the variables measured.

Pattern of food consumption during the experiment

The peak average daily consumption of about $4\frac{1}{2}$ gal. of whey/pig was reached during approximately the 25–27th weeks of life. Apart from the first 3 weeks of the experiment, however, the average daily intake of dry matter by the meal plus whey-fed pigs was appreciably below that of the all-meal fed control animals.

DISCUSSION

The slower rate of growth but better carcass grading of the whey-fed pigs as compared with the all-meal fed animals in the present trial is in general agreement with the results obtained in the earlier trial at the Cow and Gate farm in which similar comparisons were made (1). Contrary to the present results, however, in the earlier trial the whey-fed pigs utilized their food more efficiently than the all-meal fed control animals. The over-all performance and carcass grading of the pigs in the present experiment were, however, considerably better than in the earlier experiment. As previously mentioned, a similar

improvement in performance since the first experiments on the Cow and Gate farm were completed was also observed in another more recent trial (2). This improvement in general performance is probably associated in part with the breed improvement work continuously in progress in the East Farm herd and in part with a number of improvements made in the nutrition, housing and management of the experimental pigs.

Another factor probably responsible in part for the better commercial grading of the all-meal fed pigs in the present trial as compared with those in the earlier trial of Braude *et al.* (1) was the reduction in the maximum daily meal allowance given to the pigs (6 lb./pig compared with 6½ lb./pig in the earlier experiment). This reduction was made following the results obtained by Braude *et al.* (2) showing that under the conditions prevailing at East Farm, a maximum daily meal allowance of 6½ lb./pig was too high in so far as it had an adverse effect on carcass grading.

The growth curves in Fig. 1 show that there was a tendency for both the all-meal fed and whey-fed groups to increase slightly their rate of growth as they got older. This was contrary to the results obtained in earlier trials (4) in which the growth rate of the pigs fed whey tended to fall off as they got older, due, it was considered, to the pigs' having insufficient capacity to consume enough whey to enable them to maintain at least a constant rate of growth. This difference in results was probably, at least in part, associated with the very appreciably better over-all utilization of the food consumed by the pigs in the present experiment as compared with the earlier trials.

Some indication of the value of whey as a replacement for meal in this experiment is obtained from the mean feed efficiency values in Table 4 for the all-meal fed and whey-fed pigs. These figures show that 1 gal. of whey replaced approximately 0·7 lb. meal, which compares with the corresponding figure of 0·8 lb. meal given by Braude *et al.* (1). As previously mentioned, the performance of the control pigs was, however, not satisfactory in this latter trial, and it was considered that under average conditions the replacement value of whey might be somewhat lower.

While the whole fattening period of both the all-meal and whey-fed pigs is taken into consideration in arriving at the figure of 0·7 lb., any estimation of the cash value of whey should also take into account the slower growth rate with consequent reduced output of animals, the lower dressing percentage and the better carcass grading of the whey-fed pigs compared with the all-meal fed pigs.

The age at which the whey-fed pigs reached their peak average daily consumption of whey, namely approximately during the 25–27th weeks of life, agrees with the results of the second of the two trials reported by Braude *et al.* (1), and with the results obtained by Braude *et al.* (4). The peak consumption in the first of the two earlier trials occurred some 6–8 weeks sooner, however. This particular experiment was carried out during the winter, whereas all the others, including the present experiment, were run during the summer months, suggesting, as discussed by Braude *et al.* (4), a possible effect of season on age at which peak whey consumption is attained.

The general level of whey consumption, as related to age of pig, and the average peak daily intake tended to be slightly lower in the present trial than in all the above-mentioned earlier trials (with the exception of the second of the two trials reported by Braude *et al.* (1)). As a result of the appreciably better utilization of the whey consumed, however, the rate of growth of the pigs fed meal plus whey in the present experiment compared very favourably with those obtained in the earlier trials. The lower daily intake of dry matter

by the whey-fed pigs at practically every stage of the fattening period, compared with that of the animals fed meal only, shown in Table 6, is in agreement with the findings of Braude *et al.* (1), and clearly demonstrates the main reason for the slower growth rate of the former, taking as they did, about 20 days longer to reach bacon weight.

The results obtained in this experiment show that under the conditions prevailing in the Cow and Gate piggery there was no advantage in continuing to feed oxytetracycline after 130 lb. live weight either to the all-meal fed pigs or to those fed meal plus unrestricted amounts of whey. Whereas some all-meal feeding trials have been reported giving similar results to those obtained here, in others omission of the antibiotic halfway through the fattening period resulted in an adverse effect on the performance of the pigs. The reasons for these diverse results are at present obscure and are likely to remain so until the precise mode of action of antibiotics in promoting growth in pigs is elucidated. In considering these results, it should be noted that at present there is no experimental evidence, under the conditions prevailing on the Cow and Gate farm, showing the effect of antibiotics fed during the first part of the fattening period. Account should also be taken of the fact that all the pigs used had access to creep pellets containing the antibiotic penicillin from 3 weeks of age. Further experiments to investigate some of these factors are now in progress.

SUMMARY

1. Results are reported of an experiment with fattening pigs carried out under commercial conditions, to obtain further information on the comparison of all-meal feeding with meal plus unrestricted amounts of whey. In addition, the effect of omitting the antibiotic, oxytetracycline, from the diet during the latter half of the fattening period was investigated.

2. There were four treatments. (i) Meal only *ad lib.* up to a daily maximum of 6 lb./pig, with oxytetracycline included in the diet for the whole fattening period. (ii) As (i) but oxytetracycline included in the diet only up to an average pen live weight of 130 lb. (iii) 3 lb. meal reduced to 2 lb./pig/day by 13 weeks of age plus unrestricted amounts of whey with oxytetracycline included in the basal meal for the whole fattening period. (iv) As (iii) but oxytetracycline included in the diet only up to an average pen live weight of 130 lb.

There were five pens of nine group-fed pigs on each treatment, involving a total of 180 pigs. The pigs were on experiment from approximately 8 weeks of age to bacon weight. Comprehensive carcass measurements were made on all pigs.

3. The meal plus whey-fed pigs grew significantly slower and utilized their food significantly less efficiently than those fed meal only. They also had a significantly lower dressing percentage, but longer and less fat carcasses than the all-meal fed animals. The results are discussed in relation to those obtained in earlier experiments completed under similar conditions at the Cow and Gate farm.

4. Comparison of the efficiency of food utilization results of the all-meal and meal plus whey-fed pigs showed that 1 gal. of whey replaced approximately 0·7 lb. of meal.

5. The results showed that omission of antibiotic from the diet of either the all-meal or meal plus whey-fed pigs after they reached 130 lb. live weight, had no significant effect on growth rate, efficiency of food utilization or any carcass measurements of the pigs.

We wish to thank the Director of Messrs C. and G. Prideaux, Ltd., Bacon Curers, Motcombe, Shaftesbury, Dorset, for permission to take carcass measurements of the experimental pigs, and we are greatly indebted to Messrs K. Lawrence and P. Prideaux who most conscientiously took these measurements.

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HEAT RESISTANCE OF LACTOBACILLI FROM ENGLISH CHEDDAR CHEESE

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Milk containing no viable lactobacilli was essential for making the experimental cheeses to be described by Mabbitt, Chapman & Sharpe (1). It was to determine conditions of heat treatment satisfactory both to the bacteriologist and to the cheesemaker that the following work was done. At first it was necessary to know the extent of variation in heat resistance both between and within the species of lactobacilli present in Cheddar cheese. Representative strains were therefore tested in the laboratory to obtain such information: this work forms part I of the paper. With this knowledge available, it was possible to carry out further work using a commercial pasteurizer. These studies are described in part II, throughout which the finding of no surviving lactobacilli in 5 l. of milk was taken as the bacteriological standard of effective heat treatment.

I. LABORATORY-SCALE HEAT TREATMENT

Methods

Strains of lactobacilli tested

The following strains of 'low-temperature' lactobacilli, all originally isolated from milk or cheese and characterized biochemically and serologically (2, 3), were used: *Lactobacillus brevis* B 188, T 21, V 7; *L. casei* A 121, A 148, B 142, H 10; *L. plantarum* C 106, H 268, L 50; unclassified homofermentative strains A 41, A 101, K 44. A strain of *L. plantarum* var. *rudensis*, RS 5, previously isolated from a rusty spot cheese, was also used.

Determination of heat resistance

One ml. of a tomato-juice glucose broth (TGB) culture of the strain under test was mixed with 9 ml. skim-milk; in the early tests only, the number of viable organisms in this suspension (A) was determined. Then 0·1 ml. of A was inoculated, in triplicate, into 5 ml. skim-milk (B) held at the test temperature in a thermostatically controlled ($\pm 0{\cdot}1°$ C.) water-bath. These 5 ml. quantities of milk, contained in tubes of $\frac{3}{4}$ in. diameter, formed columns only about $\frac{1}{2}$ in. high, and inoculations were made using a syringe fitted with a fine needle: under such conditions, tested by adding an inoculum of methyl violet to the milk, instant thorough mixing of the inocula was ensured. The tubes B were kept in the water-bath for 10 sec., being shaken throughout. One ml. was then quickly withdrawn and immediately mixed with 9 ml. of quarter-strength Ringer's solution (C) at room temperature. Triplicate inoculations, using 0·1 ml. of A, were made also into 5 ml. quantities of milk at room temperature, and 1 ml. amounts similarly transferred to blanks of 9 ml. Ringer's solution (D). Viable counts were made on acetate agar (4), using the technique of Miles & Misra (5). The plates were incubated for 3 days at 30° C. in an atmo-

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sphere of 90% hydrogen and 10% carbon dioxide. Comparison of counts for tubes C and D gave a measure of the bactericidal effect of the particular temperature/time conditions of the test.

Results

The values calculated from counts on the tubes compared well with (though usually slightly lower than) those on the initial dilutions A. It was thus established that rapidly ejecting the cultures through the fine needle of the syringe did not separate chains or

Table 1. *Heat resistance of strains of lactobacilli from Cheddar cheese, using a laboratory pasteurization test*

		Bactericidal efficiency (% killed)				Bactericidal efficiency (% killed)	
Species	Strain	140° F./ 10 sec.	150° F./ 10 sec.	Species	Strain	140° F./ 10 sec	150° F./ 10 sec.
L. casei	H 10	61	100	*L. plantarum*	H 268	44	100
	A 121	0	100		L 50	0	100
		18	97			44	99·9
		0	98			38	—
	A 148	0	100		RS 5	58	100
	B 142	0	99·1			84	99·6
						16	—
L. brevis	T 21	6	100		C 106	0	100
		0	99·9				
		22	—	Unclassified streptobacteria	A 41	0	99·9
	V 7	13	100			24	99·3
	B 188	28	99·7			29	98
					K 44	7	100
					A 101	0	100

clumps of bacteria. Table 1 is a summary of the results for all strains tested. At the lower temperature, marked differences in heat resistance were found, even in the same strain on different occasions. These differences largely disappeared following an increase in temperature, however, and exposure to a temperature of 150° F. (65·6° C.) for 10 sec. killed all, or almost all, of the organisms of every strain.

II. LARGE-SCALE HEAT TREATMENT

Methods

Strains of lactobacilli tested

The following strains, all of which had come from milk or cheese[2,3] were used: *Lactobacillus brevis* A 142, A 160, B 146, B 155, B 188, C 101, D 127, D 162, D 210, K 46, T 21, V 7; *L. casei* A 121, A 148, B 142, B 163, C 193, H 10; *L. plantarum* A 164, A 168, AB 41, C 106, H 268, K 50, L 50, O 55, RS 5, W 60; unclassified streptobacteria A 41, A 101, A 150, AC 10, D 45, K 44, T 6, T 58, V 4, V 5.

Organisms were grown in yeast-extract glucose litmus milk (YGLM), and in each experiment the inoculum was such as to give a final concentration of about 10^4/ml. in the raw milk tank of the pasteurizing plant. To ensure even distribution of the test bacteria in the raw milk, one pint was withdrawn and the YGLM inoculum mixed with it; this relatively large volume was then inoculated into and well mixed with the rest of the raw milk.

Five experiments (A–E) were made with the plant operating at 160° F. (71·1° C.), then three (F–H) at 155° F. (68·3° C.), as follows: (A) with a single unclassified streptobacterium; (B) with four strains, one from each species; (C) with fifteen strains, comprising *L. brevis* (6 strains), *L. casei* (2), *L. plantarum* (4) and three unclassified streptobacteria; (D) with a further fifteen strains: *L. brevis* (4), *L. casei* (3), *L. plantarum* (3) and five unclassified streptobacteria; (E) with twenty-five strains: *L. brevis* (7), *L. casei* (6), *L. plantarum* (6) and six unclassified streptobacteria; (F) using a single *L. casei* strain; (G) with three strains, representing *L. brevis*, *L. casei* and *L. plantarum*; (H) with thirty-two strains: *L. brevis* (9), *L. casei* (6), *L. plantarum* (9) and eight unclassified streptobacteria.

Sterilization and operation of the pasteurizer

The experiments were carried out with an A.P.V. HX-type heat exchanger in the N.I.R.D. Dairy. This plant, and the method of its sterilization and operation, have been described by Williams, Franklin, Chapman & Clegg (6). In the present work, however, the final steam heating section was used as a holding section.

Conditions of heat-treatment

It has been found during a long-term study of the numbers of lactobacilli in cheese milk in a large number of factories, now in progress in collaboration with the National Agricultural Advisory Service, that exposure to a temperature of 160° F. (71·1° C.) for 15 sec. usually killed all the lactobacilli present in the raw milks, whereas survivors occurred when lower temperatures or shorter times of heat-treatment were used. The construction of the plant used in the present investigations was such that it was convenient to use a *mean* holding time of 17 sec. (which approximates to a *minimum* holding time of 15 sec. in commercial use). It was decided to keep the time of treatment constant and to vary the temperature for various intensities of pasteurization.

Samples for bacteriological examination

(*a*) *Raw milk.* Samples of the milk in the holding tank were taken, by pipette, before and after inoculation of the test organisms and viable counts made.

(*b*) *Heat-treated milk.* One of the bleed valves was aseptically detached and in its place was connected a sterile 10 l. aspirator bottle provided with a tubulure near its base. From the tubulure ran a short rubber tube, connected to a piece of glass tubing fixed through the top of a small bell-jar. The apparatus was designed to collect aseptically a large volume (usually about 6 l.) of heat-treated milk, which could then be dispensed in suitable quantities in the laboratory. This procedure was not only more convenient, but also greatly reduced the risk of contamination during sampling in the dairy.

From the large bulk of heat-treated milk one 2·5 l. volume and six 500 ml. quantities were dispensed; one 500 ml. portion was further subdivided into 5×50 ml. and 5×5 ml., and the remainder discarded. These sixteen quantities were incubated for 1 month at 30° C., then streaked on to acetate agar medium to determine in which containers growth had occurred. By reference to probability tables (Hoskins (7)) the most probable number (M.P.N.) of lactobacilli in 5 l. of the treated milk could be found. To prove that lack of growth in the treated milk was actually due to the heat treatment and not to the inability of the organisms to remain viable in the milk for a month at 30° C., sterile milk was inoculated with a mixture of the lactobacilli used in the tests, to give a final concentration

of about 1 organism/10 ml. Counts were made on acetate agar to determine the actual concentration of viable lactobacilli before incubation. Five × 100 ml., 5 × 10 ml. and 5 × 1 ml. volumes of this suspension were incubated for 1 month at 30° C., then the presence of viable organisms was determined by streaking an inoculum from each bottle on to acetate agar. Growth resulted on all the plates inoculated from the 100 ml. volumes, on 4/5 from the 10 ml. volumes and on 3/5 from the 1 ml. volumes. From probability tables (7) it was found that the M.P.N. was 2·8/10 ml. This compared well with the original concentration of 1·24/10 ml., and proved the suitability of the technique.

Results

Exposure to a temperature of 160° F. (71·1° C.) for a mean holding time of 17 sec. was effective in all five tests (A–E) for all the lactobacillus strains tested, in that the viable counts were reduced by a factor of the order of 10^7, namely, from about 10^4/ml. to less than 1 in 5 l. With the plant running at 155° F. (68·3° C.), however, although no growth occurred in tests F and G, growth subsequently occurred in the 2·5 l., in two of the 500 ml. and in one of the 50 ml. volumes (M.P.N. 7 in 5 l.) on the occasion when thirty-two strains were used (test H).

DISCUSSION

In the laboratory tests, strains varied markedly in their resistance to exposure to a temperature of 140° F. (60° C.) for 10 sec., whereas treatment for the same time at 150° F. (65·6° C.) resulted in an average kill of 99·55%. Slatter & Halvorson (8), also using laboratory tests, noted that the heat resistance of lactobacilli varied among strains and was not associated with biochemically defined species. Most of their cheese lactobacilli were destroyed by heating at 160° F. (71·1° C.) for 15 sec.

The experiments done using the commercial pasteurizer set to give a heat treatment of 155° F. (68·3° C.) for a *mean* holding time of 17 sec., gave kills of 100, 100 and 99·99999%. With the temperature of treatment increased by 5° F. (2·8° C.), however, 100% kills were obtained on five separate occasions. These figures agree well with predictions from the less precise laboratory results.

As milk treated at 160° F. for 17 sec. mean holding time in the heat exchanger behaved normally in the cheese vat and gave normal cheese, these conditions have been adopted for rendering cheese milk free from lactobacilli in further cheesemaking experiments.

SUMMARY

The heat resistance of strains of lactobacilli previously isolated from Cheddar cheese was determined using a laboratory pasteurization test and a commercial pasteurizer.

The laboratory tests showed variations in heat resistance at 140° F. for 10 sec. which were not correlated with species, but at 150° F. for 10 sec., an average of 99·55% destruction occurred with all strains.

With the commercial pasteurizer, heating at 155° F. for 17 sec. caused a 99·99999–100% destruction, whilst heating at 160° F. for 17 sec. destroyed 100% of all strains of lactobacilli tested in every case.

We wish to thank Miss H. R. Chapman and Miss A. J. W. Harrison for operating the pasteurizer.

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MICROSCOPICAL OBSERVATIONS ON CHEDDAR CHEESE AND CURD

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(With 4 Plates)

Many have speculated concerning the fine structure of cheese, but little definite evidence in the form of photographs has been published, and the few pictures which are available do not show the detailed structure of the curd clearly nor can anything be deduced from them concerning the distribution of bacteria in the curd and the way they may be embedded therein. However, Koestler (1) has published a photograph of many colonies as dark areas in a stained section of Emmenthal cheese under low-power magnification. He also showed a small colony of bacilli and another of streptococci at a high magnification. Curd granule boundaries were apparent at the lower magnification. Similar granule boundaries were observed in Gouda and Edam by Hekma (2).

More recently, Bourne & Hawley (3) have stated that the cheese mass consists of 'lobules with a discrete boundary between adjacent ones'. They noted variations in size and intensity of staining of fat droplets and other details in different types of cheese.

King (4) has also made a number of observations and has recently published beautiful photographs showing the spongy structure of freshly made cheese curd.

Our own interest in this field arose in the first place because of the difficulty of securing reproducible counts of the living bacteria in cheese for the study of ripening processes. When an inconveniently large sample (e.g. six borings) was taken fairly reproducible results were obtained, but as the size of the sample was diminished so the variation increased until with very small samples large fluctuations were observed. For example, Table 1 shows counts of lactobacilli obtained by plating small samples on the selective medium of Mabbitt & Zielinska (5). It appeared that the dispersion of the bacteria was heterogeneous, and microscopical examination of sections would be worth while. This also provided an opportunity to study the microscopic structure of the curd itself.

Table 1. *Variations in counts of lactobacilli in small samples of cheese*

(Samples weighing 0·014 g. were obtained aseptically by means of a small glass cylinder.)

Cheese no.	Plate counts on acetate agar (5)		
	Sample *a*	Sample *b*	Sample *c*
L 1	<500	4,000	<500
L 2	>50,000,000	>40,000,000	>50,000,000
L 3	3,300,000	<500	>50,000,000
L 4	<500	<500	>40,000,000
L 5	3,750	>500,000,000	16,250

EXPERIMENTAL MATERIAL AND METHODS

Five cheese were made in the Experimental Dairy of this Institute by the normal methods for Cheddar, with variations in scalding rate and in the force applied in the press. One was made from pasteurized milk and the remaining four from raw milk.

A few samples in the form of small blocks were sectioned on the freezing microtome without previous treatment, but the majority (as indicated in legends below the photographs) were fixed in formol saline (10 ml. 40% (w/v) formalin, 90 ml. water, and 0·85 g. sodium chloride) for at least 10 days at room temperature. Sections were then cut with a freezing microtome, floated on to a thin layer of 0·25% gelatin solution to allow them to flatten, and dried at 37° C. for 2 hr. in an atmosphere containing formaldehyde vapour. No serious shrinkage was observed during drying. The microtome was set to give a thickness of 5 μ, but owing to the springiness of the curd the sections were not uniform. The thickness recorded is therefore only approximate. Of the various stains tested Borrel's methylene blue (6) (prepared by boiling methylene blue with silver oxide) was the most suitable for demonstrating both curd structure and bacteria. For showing bacteria at greater contrast Broadhurst & Paley's stain (7), designed for milk smears, was used. These were the only stains used in preparing the specimens for the photographs in Pls. 1–4. Several fat stains were also tried. Oil red 4 B gave bright coloration in good contrast with the blue of casein stained with methylene blue. However, in such sections the bacteria could not easily be seen. The fat stain merely confirmed that the voids appearing in sections stained with methylene blue were filled with fat.

Experiments on wax embedding were carried out and polyethylene glycol ('Carbowax') was sometimes successful. Embedding in paraffin wax was used successfully by Hiscox (8), but was avoided here to minimize the risk of removal, during the dehydrating procedures, of any organisms which might not be firmly fixed to the casein.

RESULTS

(1) *Effect of fixation*

Formalin fixation has previously been used for cheese, but since there is a marked effect on the structure of curdled milk when formalin is applied before syneresis (9) alternatives were tried. None of several other fixatives was successful, but it was possible to cut unfixed cheese with the freezing microtome. The majority of the sections prepared in this way appeared satisfactory until they were compared with sections from fixed cheese when it was observed that the latter usually showed more fine detail. It appeared as though the fine protein fibres in the unfixed cheese were too delicate to withstand the treatments of floating on to slides, staining and re-drying. The difference is illustrated in Pl. 1 A and B. In order to test whether the fine fibres seen in some of the fixed sections were the remains of a more cellular or foamy structure altered by formalin, the rates of drainage of fat at 37° C. from fixed and unfixed pieces of cheese were compared. Fat drained slightly more quickly from the unfixed cheese suggesting that fixation had, in fact, stabilized whatever cellular structure there may have been in the original cheese.

(2) *Effect of embedding*

Carbowax (polyethylene glycol) was found to be the most suitable embedding material. Frequently quite good sections could be obtained from the embedded cheese (Pl. 2 G),

but sometimes the results were inferior, possibly owing to the long period at 37° C. necessary for penetration by the wax. These sections showed less fine detail than the frozen ones (cf. Pl. 1 C, D).

(3) *Appearance of curd and cheese at different stages of ripening*

The sections stained with methylene blue gave the impression of an irregular three-dimensional protein network of indefinite extent. Pictures of this kind arose out of the necessity of cutting thick sections (since thin ones were too fragile), and it was possible to photograph them because of the intense staining of the protein of the curd with methylene blue which caused the registration on the photographic plate of portions of sections above and below the plane of focus. The resulting three-dimensional effect probably gives a truer picture than would an ideal section. Experiments with fat stains gave the impression that the meshes of the solid network were entirely filled with fat. This is perhaps not true since aqueous fluid amounting to 10–20% of the weight of the cheese can be separated by intense pressure (200 lb./sq. in.) applied after grinding with sand. The need for grinding and for intense pressure and the absence of obvious moisture droplets in the curd all suggest that the aqueous fluid is more finely dispersed than the fat.

Considerable variations were sometimes noticed between curds which were expected to be identical. Some variation may have originated in imperfections in technique, as, for example, the escape of fat from very thin sections, or the softening of inadequately fixed portions of protein when contact with the dilute gelatin solution was too prolonged as a result of slow drying. However, the uncertainty in interpretation was diminished by a view of the results as a whole. The gradual nature of the changes observable in a series of sections taken from the same cheese at different stages of ripening, together with the regularity in difference between one cheese and another throughout the ripening period, suggested that most of the differences originated in the cheese themselves. Thus the curd which showed the most fine structure at the earliest stage retained most fine structure as ripening proceeded. In spite of the fluctuations each of several cheeses used for the study of ripening showed a loss of fine structure as ripening proceeded. This was most marked in cheese no. 1 of which the first and last pictures are shown (Pl. 2 E, F). The ripened cheese was so soft, even after fixation, that it was necessary to cut the sections at a thickness of 10 μ. Although the changes were less dramatic in cheese no. 4 this showed the fine structure and the disposition of micro-organisms to the best advantage. The series is therefore reproduced in full (Pl. 2 G, H; Pl. 3 J, K, L, M).

(4) *The effect of variations in cheese-making technique*

It was not possible to correlate the small differences observed with the techniques of making.

(5) *Disposition of micro-organisms*

Diplococci can clearly be seen in Pls. 2 and 3, and some of them are surrounded by haloes as though they had digested the casein or modified its staining properties. However, as the staining of the casein is darker in the riper cheese the micro-organisms are more difficult to distinguish. They may more easily be seen in slides by the method of Broadhurst & Paley. Two such examples are illustrated in Pl. 4 N and O, but there is little of interest to add. One sees merely cocci and diplococci scattered sporadically amongst the casein. As can be seen the distribution was very irregular, but was more so than

appears from the photographs since fields practically devoid of micro-organisms could be found.

The bacteria do not normally occur inside the fat droplets, and when, as rarely, in the photographs they appear to do so they are probably just above or just below the fat in a thin layer of 'casein', or possibly they may have been moved during sectioning. The rarity with which they may be found inside the larger 'knots' of casein is also remarkable. This can only be demonstrated on slides in which the casein itself is not too deeply stained, and especially by Broadhurst & Paley's stain. It seems that there is a tendency for the bacteria to congregate at the fat droplet surface.

Colonies were rarely seen, but by searching the whole of a section (about 1 cm.^{2}) under the low power a colony could occasionally be found. All those colonies so far discovered existed in cracks or holes. An example is illustrated in Pl. 4 P, which shows part of a section under the $\frac{2}{3}$ in. objective. The dark zones indicate highly stained bacteria lying in the cavity. The dark lines around the larger fat globules, however, do not indicate bacteria as was proved by an examination of the slide under high power. The lines are probably an effect of refraction resulting from photography of a dry section. A portion of this colony examined under oil immersion showed both rods and cocci with various degrees of staining (Pl. 4 Q). It was also apparent from an examination of several fields that the walls of the cavity were lined with an almost continuous film of bacteria (bottom left-hand corner), but the boundaries between 'casein' and fat contained only an occasional organism (rest of left-hand edge). The right-hand side of photograph Q shows a mass of micro-organisms in the cavity with no background of curd. The remainder appears to show the bacteria growing over a small knob of curd which probably projected into the cavity.

DISCUSSION

Fresh cheese curd contains about one third by volume of fat and two thirds of wet relatively homogeneous protein which forms a continuous phase. It is obvious that thin sections of a mixture of one third transparent colourless spheres and two thirds coloured opaque interstitial substance would present a picture consisting of white or faintly coloured circles on a darker background. Cheese sections deviate from this ideal picture, and possible reasons for this are as follows.

As soon as the curd is allowed to settle and whey is drained off, and especially during cheddaring, considerable flow or deformation occurs. The soft fat globules will easily change in shape, but it is reasonable to suppose that the 'casein', though tougher, will suffer some damage and indeed will be torn at points of greatest stress. These points will occur in thin walls of 'casein' between adjacent fat droplets in regions subject to flow. The forces of surface tension on the thin edges of torn walls must be enormous. Thus thin sheets between larger masses of 'casein' will tend to form threads, whilst torn sheets with a 'free' end will form thicker spikes and knobs. This process will also produce coalescence of fat globules.

The collapse of sheets into threads and of threads into knobs will be aggravated in a section subjected to the softening action of water in the uncurling treatment, unless the protein has been fixed. On the other hand, it is unlikely that fixation with formalin will produce threads and sheets from knobs. It seems reasonable therefore to assume that the finest structures demonstrated are nearest to those actually existing in the curd.

It may well be that stresses remain in the interior of a pressed cheese, and the weight of the cheese itself produces stresses, and, in the absence of bandages, visible strains as well. Thus, as the curd protein softens during ripening individual 'casein' walls between fat droplets will tear. This tendency will be increased by the hardness of the fat at the lower temperature of ripening and, on the other hand, it will be resisted by the increase in toughness of the protein caused by drying. Thus the picture is complex and a ripened cheese may, in fact, be little different in mechanical structure from the original curd, though the protein may be degraded chemically. The cross-linking by formaldehyde will, however, be less effective when the protein is degraded, i.e. fixation will be less effective, and it may be that even when differences do not occur in micro-mechanical structure between curd and ripened cheese the sections will appear different because those from the ripe cheese are less resistant to treatment with aqueous solutions.

Whether the network actually breaks before the cheese is sectioned remains an open question. If it does it must influence the texture and body of the cheese.

The occurrence of colonies only in crevices is remarkable. The streptococci, which are numerous at the milling stage, do not increase greatly after salt is added. Thus the individual bacteria do not grow into colonies. Possibly, under other conditions, micro-organisms capable of forming colonies in the body of the curd would be numerous enough to be found in the very small sample represented by a few sections. The possibility that many organisms can only grow in the crevices would be sufficient to explain the differences in cheese type caused by different degrees of milling and different treatments after the pitching stage.

SUMMARY

1. It is possible to study the structure of cheese curd and the disposition of micro-organisms therein by microscopical observation of sections cut on the freezing microtome after fixation in formalin.

2. In the small number of cheese examined bacterial colonies were found only in crevices.

3. As ripening proceeded the finer protein structures tended to disappear.

4. The distribution of bacteria in cheese curd is markedly uneven.

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EXPLANATION OF PLATES

Except where otherwise stated, all sections were fixed, cut on the freezing microtome and stained with Borrel's blue as described in the text. Magnification (except P) about 800 diameters.

Plate 1

A. Cheese 4 weeks old. B. As A, but not fixed. C. Fresh curd before milling and salting. D. As C, but fixed and embedded in Carbowax.

Plate 2

E. Fresh curd (as C, Pl. 1) (the circular zone with dark central staining was not identified). F. Cheese from curd used for E after 16 weeks' ripening. G. Fresh curd (as C, Pl. 1), but wax embedded. H. As G, but after 8 days' ripening (normal fixation, etc.).

Plate 3

J. As G (Pl. 2), but after 4 weeks' ripening (normal fixation etc.). K. As G (Pl. 2), but after 8 weeks' ripening (normal fixation etc.). L. As G (Pl. 2), but after 12 weeks' ripening (normal fixation etc.). M. As G (Pl. 2), but after 16 weeks' ripening (normal fixation etc.).

Plate 4

N. 16-week-old cheese. Broadhurst and Paley's stain. O. Another 16-week cheese, stained as N. P. 16-week cheese at low magnification showing colonies in a crack, stained as N. Q. Part of P at high magnification showing cocci, rods and faintly-stained casein (upper left-hand corner).

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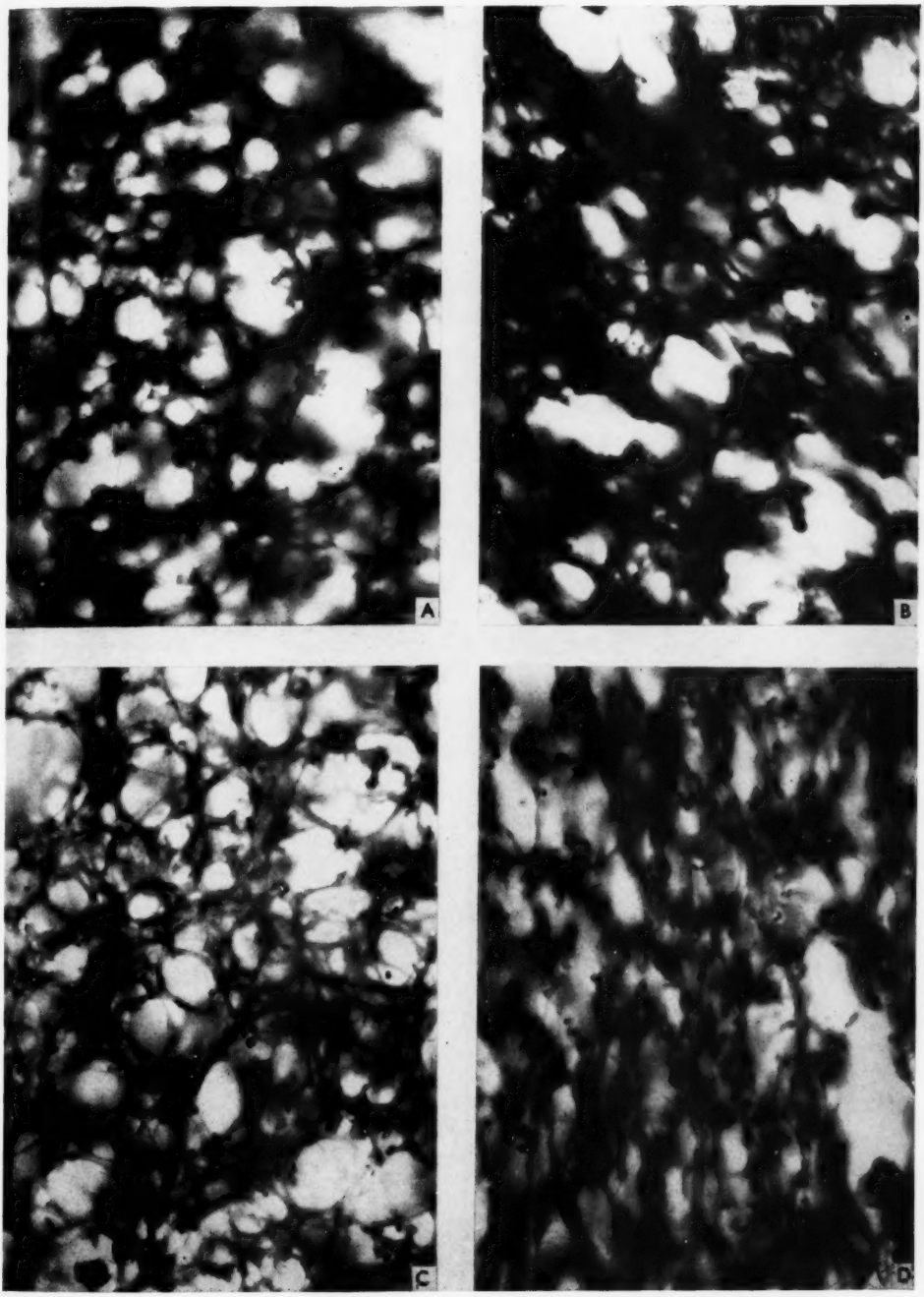
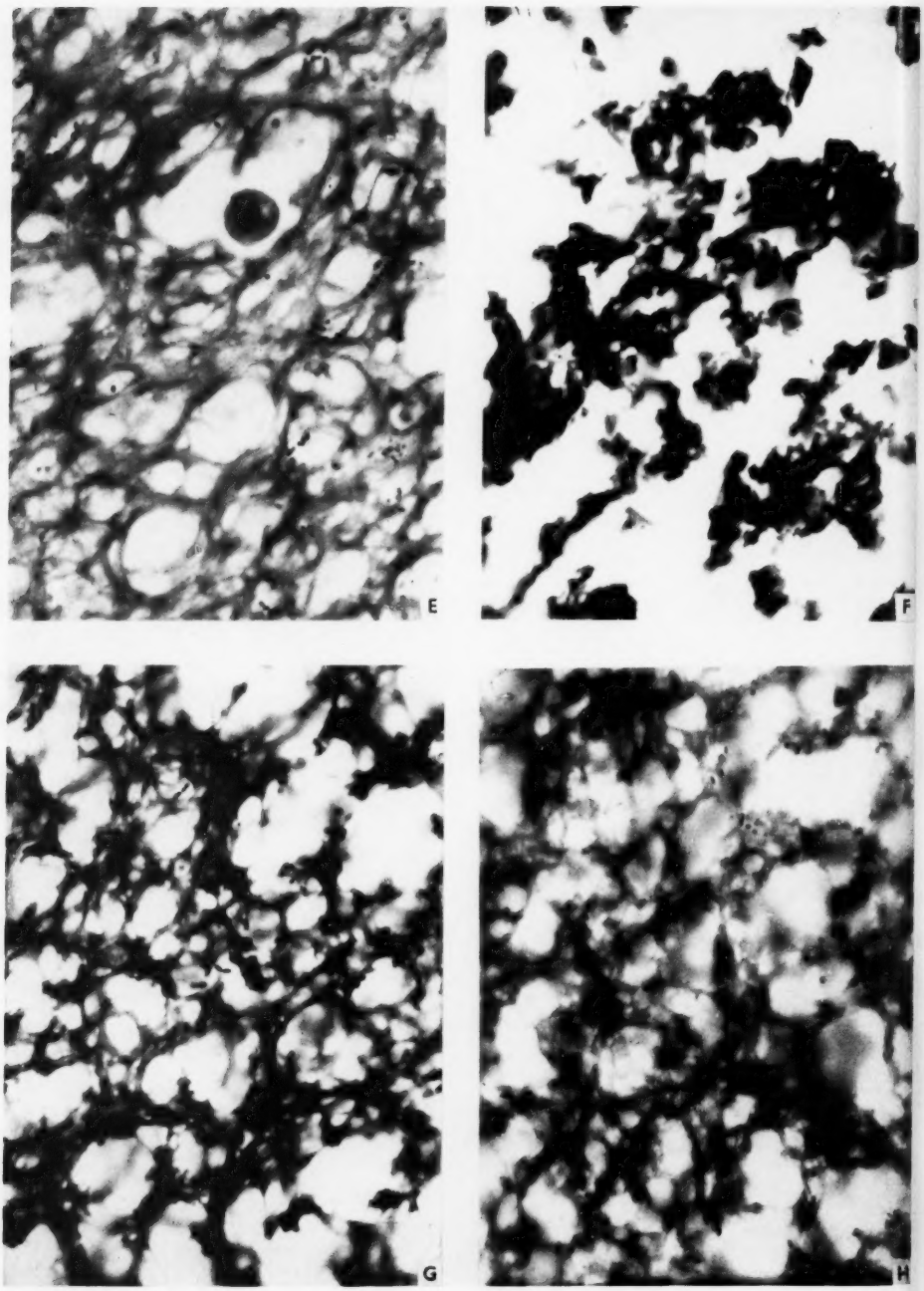


DEAN, BERRIDGE & MABBITT—Microscopical observations on Cheddar cheese and curd

(*Facing p.* 82)



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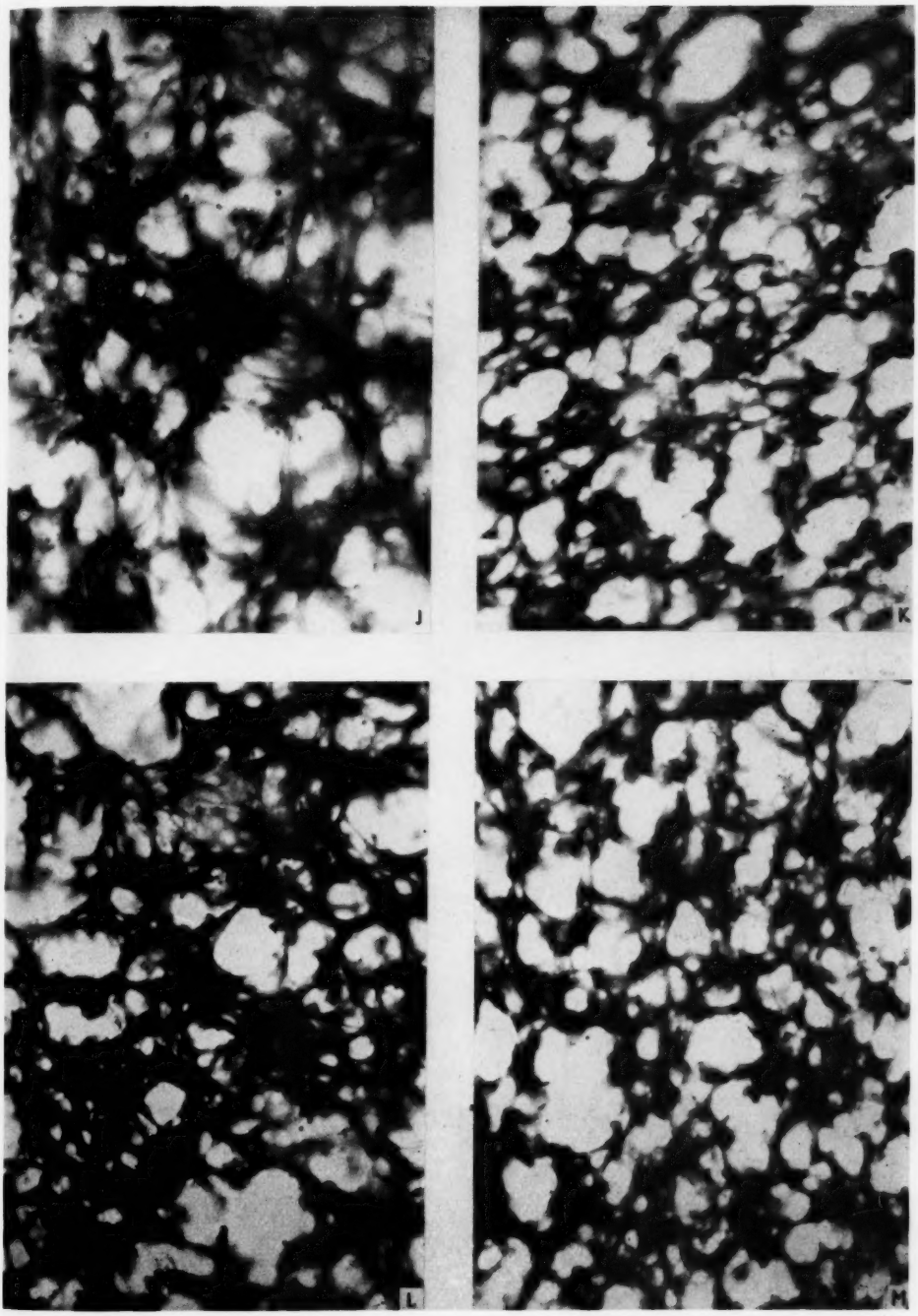
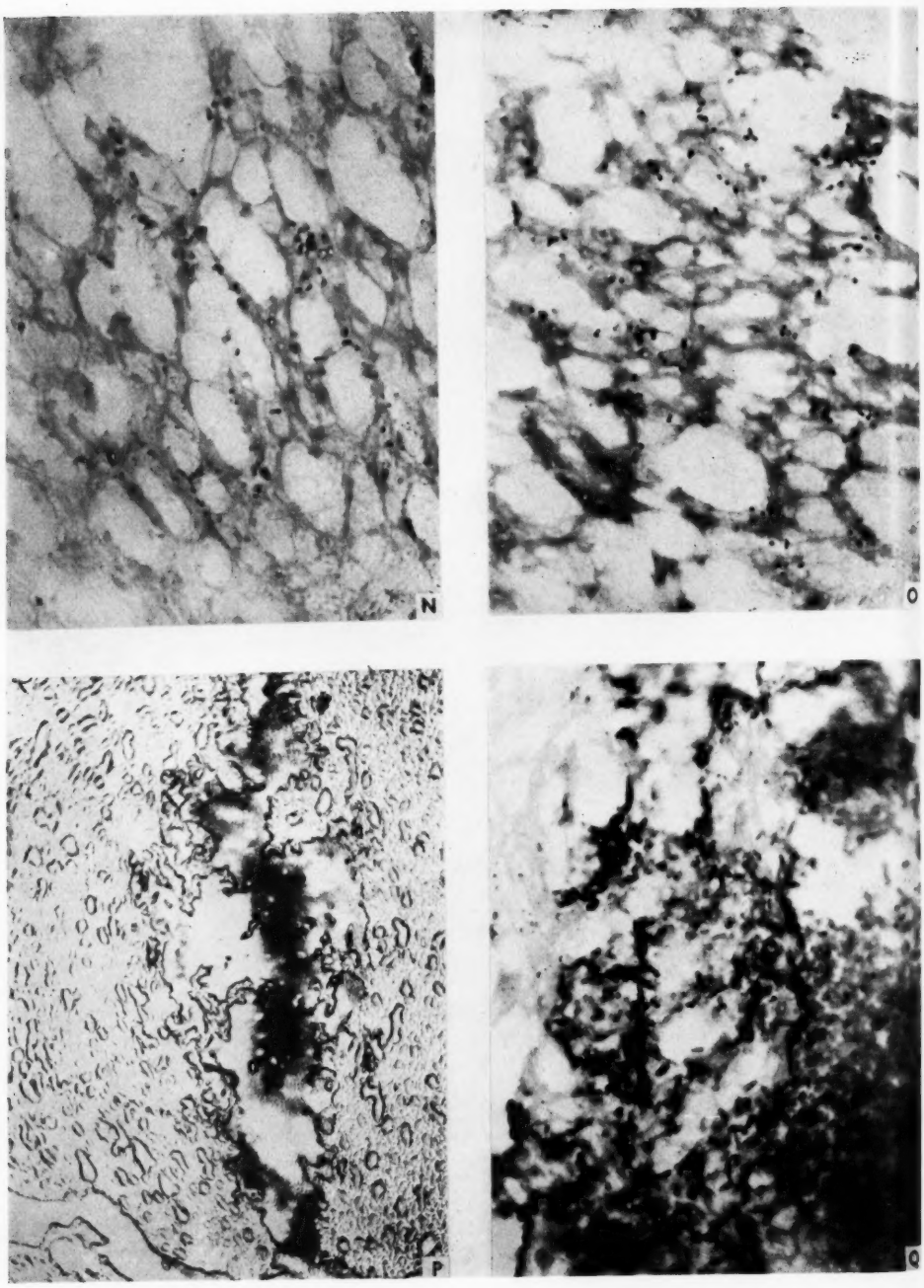


DEAN, BERRIDGE & MABBITT—Microscopical observations on Cheddar cheese and curd



DEAN, BERRIDGE & MABBITT—Microscopical observations on Cheddar cheese and curd

THE ESTIMATION OF THE TOTAL SOLIDS AND SOLIDS-NOT-FAT OF MILK FROM THE DENSITY AND FAT CONTENT

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When British Standard 734, 'Density hydrometers for use in milk', was issued in 1937 (1), density at 20° C. was adopted as a basis for hydrometry in place of specific gravity at 60° F. Also, to ensure reproducible values for density, it was specified that milk samples should be pre-warmed to 40° C. to remove any Recknagel contraction by restoring the fat to the liquid state—a treatment first applied by Bakke & Honegger (2).

The following formula was specified in B.S. 734 for the calculation of the total solids percentage (T) from the fat percentage (F) and the density hydrometer reading at 20° C. (D):

$$T = 0{\cdot}25D + 1{\cdot}21F + 0{\cdot}66.$$

The formula was derived from Richmond's well-known formula, based on specific gravity, by adjusting for the expansion of milk and the density of water at 60° F. Richmond stated that his formula applied to samples of milk in which the fat was in the solid condition, but no correction was made in the B.S. formula for the lower density resulting from the pre-warming, since tests had shown that it 'yields results for total solids which are in satisfactory agreement with those obtained by direct gravimetric determinations' (3). Laboratories which had previously used Richmond's method would, however, get lower results by changing to the density procedure and formula. Although it was known that Richmond's method tended to give high results (4), fuller information on the accuracy of the B.S. density method was clearly desirable.

We have carried out two investigations, one in 1942–44 and one in 1948, which were briefly described in the Reports of this Institute. In the meantime, the density method was widely adopted by the creameries in this country, and interest in its accuracy was much increased by the introduction, two years ago, of an approved scheme for the regular testing of producers' milk supplies for solids-not-fat. We therefore made a further examination of our data, and this paper gives the general results which led us to propose a modification to the B.S. formulae for total solids and solids-not-fat, to improve their accuracy. The modified formulae were adopted in October 1957 in an amendment to the B.S. 734 published in 1955 (5).

EXPERIMENTAL PROCEDURE

Source of milk samples

First investigation, 1942–44

Creamery A. About 40 samples of milk, each representing the mixed evening and morning milk of a single herd, were taken on one morning each month from July 1942 till June 1944 (except in February 1943).

Creamery B. About 40 samples of mixed evening and morning milk were taken at intervals from the tipping tank on one morning each month from September 1942 till August 1944. Each sample represented 125–175 gallons of milk.

Second investigation, 1948

Samples of milk, each representing mixed evening and morning milk, were collected monthly, from February till December 1948, from about 55 herds of the Ayrshire, Friesian, Guernsey or Shorthorn breed. These samples were collected primarily for a study of the detailed composition of the milk of the various breeds.

Laboratory methods

Density and fat

Fat percentage was determined in duplicate by the Gerber method of B.S. 696:1936 (6). Checked, accurate butyrometers and pipettes were used, and the butyrometer readings were taken to 0·02% and averaged.

Density at 20° C. was determined as described in B.S. 734:1937, using N.P.L.-tested hydrometers. The readings were taken to 0·1 on the scale, and the corrections, if any, were applied. The total solids percentage was then calculated from the density formula.

Total solids

Total solids were determined gravimetrically, in duplicate, by the technique of Golding (7) which was subsequently adopted as 'Method 2' in B.S. 1741:1951 (8).

RESULTS

For each of the milk samples, the percentage of total solids found gravimetrically was subtracted from that found by the density formula to obtain the algebraic difference, which is the error in the total solids, and corresponding solids-not-fat percentages as estimated by the density procedure.

The distribution of this error, in class intervals of 0·05, is illustrated in Table 1 by the results for the typical series of herd samples taken over the two years at Creamery A.

Table 1. *Distribution of the error in the total solids and solids-not-fat percentages estimated by the density method on samples from Creamery A*

Range of error	Percentage of total no. of samples		
	July 1942 to June 1943	July 1943 to June 1944	July 1942 to June 1944
+0·18 to +0·22	—	0·4	0·2
+0·13 to +0·17	0·2	0·9	0·6
+0·08 to +0·12	1·9	6·1	4·1
+0·03 to +0·07	7·0	11·9	9·6
−0·02 to +0·02	17·6	16·5	17·0
−0·07 to −0·03	23·7	19·5	21·5
−0·12 to −0·08	21·9	19·5	20·6
−0·17 to −0·13	18·3	15·2	16·7
−0·22 to −0·18	5·9	6·3	6·1
−0·27 to −0·23	2·4	2·4	2·4
−0·32 to −0·28	0·9	0·9	0·9
−0·37 to −0·33	0·2	0·2	0·2
−0·42 to −0·38	—	0·2	0·1
No. of samples	426	461	887

For 17·0% of the total number of samples the error was within ±0·02, but positive errors greater than 0·02 occurred in only 14·5% of the samples in contrast to negative errors in 68·5%. The distribution of this series of values, and the corresponding distributions for Creamery B and the herd samples of 1948, conform closely to a normal distribution. The errors observed in the investigations may therefore be summarized in terms of their means and standard deviations, and this is done on a yearly basis, giving five series of values, in Table 2. In each of the five series the density method gave, for total solids and solids-not-fat, mean results which were lower than those obtained gravimetrically—lower by an over-all average of 0·060.

The fat and solids-not-fat contents of the milk samples examined covered the usual wide range of commercial herd samples. The mean composition of each series is included in Table 2.

Table 2. *Summary, for each year, of the milk composition and of the error in the total solids and solids-not-fat percentages estimated by the density method*

Source of samples	No. of samples	Mean fat (%)	Mean S.N.F. (%)	Error mean and S.D.
Creamery A, July 1942–June 1943	426	3·82	8·71	−0·070±0·080
Creamery A, July 1943–June 1944	461	3·80	8·64	−0·058±0·095
Creamery B, Sept. 1942–Aug. 1943	484	3·62	8·66	−0·060±0·059
Creamery B, Sept. 1943–Aug. 1944	465	3·63	8·62	−0·039±0·076
Herd samples, Feb.–Dec. 1948	589	3·74	8·74	−0·066±0·084

Table 3. *Variation in the monthly mean error in the total solids and solids-not-fat percentages estimated by the density method*

	Creamery A		Creamery B		
Month	July 1942 to June 1943	July 1943 to June 1944	Sept. 1942 to Aug. 1943	Sept. 1943 to Aug. 1944	Herd samples, 1948
January	−0·01	+0·01	−0·02	0·00	—
February	—	+0·03	−0·03	+0·02	−0·02
March	−0·06	−0·04	−0·02	−0·02	+0·02
April	−0·12	−0·09	−0·04	+0·02	−0·04
May	−0·09	−0·11	−0·06	−0·10	−0·08
June	−0·09	−0·09	−0·09	−0·06	−0·08
July	−0·07	−0·02	−0·06	−0·02	−0·06
August	−0·12	+0·01	−0·09	−0·10	−0·07
September	−0·13	−0·12	−0·09	−0·08	−0·12
October	−0·08	−0·14	−0·11	−0·11	−0·11
November	−0·04	−0·05	−0·08	−0·03	−0·10
December	−0·02	−0·07	−0·02	+0·03	−0·05

The variation in the error for samples of milk from individual herds is shown by the standard deviations for the samples taken at Creamery A and in 1948, which were in reasonable agreement at ±0·080, 0·095 and 0·084.

The errors of the density method are given on a monthly basis in Table 3. There are consistent variations in the monthly mean error, from the smallest negative, and some small positive, values (−0·03 to +0·003) in January and February to the largest negative values (−0·08 to −0·14) in September and October. These variations reflect the seasonal changes in the relative proportions of the proteins and lactose in the solids-not-fat (9).

DISCUSSION

Since the investigations have shown that the density method of B.S. 734 gives total solids and solids-not-fat percentages which, on the average, are lower by 0·06 than those found gravimetrically, the original formula should be modified to bring the average results into agreement, and to distribute the range of monthly errors equally among positive and negative values.

It is therefore recommended that the formula be modified by the addition of a constant, of 0·06, to

$$T = 0{\cdot}25D + 1{\cdot}21F + 0{\cdot}72$$

to give the best average results when the fat percentage is determined using the 11·04 ml. Gerber milk pipette as formerly specified.

The change in the capacity of the Gerber milk pipette from 11·04 to 10·94 ml. in the revised B.S. 696 of 1955, to improve the accuracy of the Gerber results, makes a second adjustment of the formula necessary to compensate for the lower fat percentage, $1{\cdot}21F$ being changed to $1{\cdot}21F \times 11{\cdot}04 \div 10{\cdot}94$, i.e. to $1{\cdot}22F$. It is therefore proposed that the following formula be adopted for current use:

$$T = 0{\cdot}25D + 1{\cdot}22F + 0{\cdot}72.$$

The corresponding formula for the solids-not-fat percentage $(T - F)$ then becomes:

$$\text{S.N.F.} = 0{\cdot}25D + 0{\cdot}22F + 0{\cdot}72.$$

The solids-not-fat percentages so calculated will give the best average agreement with those obtained by subtracting the present, improved Gerber fat percentages from the gravimetric total solids.

An abstract of this paper was submitted to the appropriate committee of the British Standards Institution, and, as already stated, the proposed formulae have been adopted (5).

SUMMARY

The accuracy of the method specified in B.S. 734:1937 for the estimation of the total solids and solids-not-fat contents of milk from the density and fat content has been assessed by direct comparisons with the gravimetric method. A total of 2425 samples of milk, mostly from single herds, were examined in five series over different years.

The percentages of solids estimated by the density method were lower, by an average of 0·06, than those determined gravimetrically. To correct for this error, and to compensate for the lower, more accurate fat percentages arising from the recent reduction in the capacity of the Gerber milk pipette, it was proposed that the density formulae be modified to $T = 0{\cdot}25D + 1{\cdot}22F + 0{\cdot}72$ and $\text{S.N.F.} = 0{\cdot}25D + 0{\cdot}22F + 0{\cdot}72$.

These formulae were adopted in October 1957 in an amendment to the B.S. 734 published in 1955.

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REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION F. MILK-BORNE DISEASE

In recent years great changes have occurred in the incidence and distribution of infectious disease. The large epidemics of the past resulting from the carriage of infection through the agencies of milk and water have almost disappeared. They have, however, been replaced, to some extent, by outbreaks due to other types of food becoming contaminated with organisms pathogenic to humans. This has followed from changes in the eating habits of the peoples in many countries, for communal food has replaced much of that previously provided for a single family. Food now available as a result of advances in methods of preservation and distribution is of great value in the nutrition of the people all over the world, but these preserved and mass-produced foods have to be manufactured under the best of hygienic conditions so as to avoid possible contamination. Skilled personnel are more than ever required for the control of food in the raw state, for the running of manufacturing plants, for the hygenic control of such plants, and for the bacteriological checking of the products during manufacture and distribution.

In many countries the hygenic quality of milk and milk products has been controlled by chemical and bacteriological tests. Some workers have suggested, however, that in view of the products that are made, even more stringent standards should be adopted. The content of extraneous matter and of bacteria significant to public health has been studied extensively by Thatcher (1) in Canada. Table 1, taken from this author's paper, is a revelation of the amount of

Table 1. *The content of extraneous matter and of bacteria significant to public health in 421 shipments of milk delivered to cheese factories*

	Shipments affected (%)
Extraneous matter*	
Rodent hairs	6·4
Bovine hairs	60·0
Manure fragments	76·0
Insects and insect fragments	38·0
Steel wool fragments	10·0
Excessive amorphous sediment (dirt)	30·0
Bacteriological†	
Colony count $>10^6$/ml.‡	74·6
Coli-aerogenes count $>10^4$/ml.	58·9
Faecal streptococci $>10^4$/ml.	31·4
Coagulase-positive staphylococci $>10^4$/ml.	35·5
Paracolon bacilli (presence only)	14·0
Salmonella	0·0
Streptococcus agalactiae§	67·0
Leucocytes $>10^6$/ml.§	33·0

* By microscopic examination of sediment from pint milk samples obtained with standard 'off the bottom' technique.
† Samples pipetted from bulk containers.
‡ These are arbitrary limits and not acceptable standards.
§ From 111 samples only.

contamination that can occur in milk. This author has discussed in great detail the functions and limitations of micro-biological standards for food as regards their desirability, design, enforcement, the presence of pathogens, the definition of specific microbial tolerances, practicability, international and administration standards, and many other aspects. Again, the study of the close relationship of infectious disease in animals to certain infections in man is of the utmost

importance. In this connexion a paper by Heisch(2) gives a very extensive review of the whole subject of the zoonoses defined in a recent publication of the World Health Organization as 'those diseases which are naturally transmitted between vertebrate animals and man'. *The Annual Report of the Ministry of Health* for 1955(3) reviews the progress made in the elimination of milk-borne disease in England and Wales. It states that, reduced to basic principles, milk-borne disease is dependent on three factors: (*a*) the amount of disease in dairy cattle, (*b*) the amount of disease in milk handlers, and (*c*) the extent to which supplies are heat-treated. At first the greatest single factor in the reduction of milk-borne tuberculosis was pasteurization, but now the time is approaching when tuberculosis will be practically eliminated from all dairy herds. Thus, this most important milk-borne disease will soon be a thing of the past in this country. Furthermore, as a result of immunization, outbreaks of diphtheria no longer occur, and the incidence of sore throat and scarlet fever has been reduced in humans with consequent reduction of the possibility of transference of infection to the cow's udder. This section of the report also discusses infections due to the presence of *Staphylococcus aureus*, *Salmonella typhi murium*, *Salmonella dublin*, and *Coxiella burneti*, and some previous outbreaks of disease due to these organisms are mentioned. Burrow(4) has given a detailed account of milk hygiene in Great Britain to the Laboratory Section of the American Public Health Association at the Eighty-fifth Annual Meeting in Cleveland. The author describes the various administrative steps that have been taken since 1885 when producers became registerable with local authorities for the first time. For a number of years only tuberculosis was regarded as a milk-borne disease, but the Milk and Dairies (Consolidation) Act of 1915 took cognizance of other diseases which can be transmitted from dairy cattle to man. Further improvements in hygiene were made effective when the Milk and Dairies Regulations of 1926, The Milk (Special Designation) Raw Milk Regulations, 1949 to 1954, and the Milk (Special Designations) (Pasteurized and Sterilized Milk) Regulations 1949 to 1953, were brought into force. The author states that: 'the picture of milk hygiene in Great Britain is not very pretty—it is a picture depicting a policy resulting from a failure to retain the best features of past organizations, while at the same time wedding them to an up-to-date organization. It must not be thought, however, that the British public are provided with a milk supply which is completely out of line with modern ideas of milk hygiene, but have come to place too great a reliance on heat treatment of milk forgetting that, while heat treatment has its origin in the need to destroy pathogenic organisms, commercial interests have turned it largely into a means of extending the period of sale-ability of milk. Instead of being a valuable adjunct to the elimination of disease in dairy cattle, and of the human beings who handle them and their products, pasteurization has unfortunately come to be used on the farm as a substitute for preventive medicine both human and veterinary'.

Leff(5) has published a book on *Recent Outbreaks of Infectious Disease*, and has described in detail outbreaks of typhoid, paratyphoid, food-poisoning and Q fever due to milk or milk products being infected with *S. typhi*, *S. paratyphi* B, Salmonellae, Staphylococci and *C. burneti*.

TUBERCULOSIS

Several important new books contain chapters on animal and human tuberculosis. Francis(6) has published a book which provides an up-to-date account of many aspects of tuberculosis, and which will be of particular value to all concerned with investigation into the veterinary and human aspects of this infection. He has provided an account of tubercular disease in all species of animals for which there is reliable information. In the first chapter of the first section, 112 pages are devoted to bovine tuberculosis, including an adequate account of the disease in man. The first section also deals with tuberculosis in animals other than bovines and contains a vast amount of information not readily available elsewhere. The final section of the book gives a comparison of the pathology and epidemiology of tuberculosis in animals and man.

Heaf(7) has edited a symposium on tuberculosis consisting of articles by fourteen contributors. The book is mainly concerned with the human aspects of the disease, but various contributors

refer to the bovine type of infection in sections dealing with the bacteriology, prevention, regulations relating to milk, infection of children, and animal tuberculosis. The chapter on bovine tuberculosis is written by Francis.

Great progress continues to be made in the eradication and control of bovine tuberculosis. So far as Great Britain is concerned, the Minister of Agriculture (8), speaking in 1957 at the annual lunch of the Royal Veterinary College, predicted that within five years or less, Great Britain would be free from bovine tuberculosis. He said that statistics showed that 90% of the population now drunk tuberculin tested, pasteurized, or sterilized milk.

The Annual Reports on Animal Health Services (9,10) give an account of the progress made under the voluntary attested herds schemes and the area eradication plans. At the end of 1956 there were nearly seven million attested cattle in Great Britain, which was about 67% of all cattle in the country. The Annual Report for 1955 (9) also gives a full description of the methods adopted.

The progress in the eradication and control of bovine tuberculosis in Australia has been examined by Gregory (11) for Australia as a whole, and for the various states, by Golding (12) for New South Wales, Webster (13) for Queensland, Chamberlain (14) for South Australia, Meldrum (15) for Tasmania, Grayson (16) for Victoria and Toop (17) for Western Australia. The progress made, and the deficiencies that existed, were fully reviewed. So far little attempt has been made to control the disease in beef cattle, and the number of dairy cows coming under control varied greatly in the different States. The author recommended an increase in the use of veterinarians in rural areas and a more complete examination of abattoir-killed meat.

Van Zwannenberg, Stewart, Harding & Gray (18) described how attention was called to the occurrence of tuberculosis neck glands in certain school children in a small market town of East Suffolk with a total population of 7400. The situation was accordingly investigated. Seven cases were known before the investigation started, but a further 12 were discovered. Of the 1360 school children, 1063 were tuberculin tested and the families of those (260) who gave a positive reaction were later also tested. The children showing tuberculin positive reactions were then related to four possible milk supplies. For each 120 gallons of milk supplied, Dairy A had 86 reactors, Dairy B 48, Dairy C 11·3 and Dairy D 11·3. Further, no less than 18 of the cases with tuberculous neck glands received their milk from Dairy A and only one had received milk from Dairy B. Dairy A was a producer-retailer of undesignated milk. When application was made for a T.T. licence, it was found that 19 out of 24 cows gave positive tuberculin reactions. It was believed, therefore, that Dairy A was the source of infection.

Nielsen (19) stated that in the county of Randers in Denmark only an occasional tuberculin reactor had been found among the cattle since 1950. The author had the impression, however, that the bovine type of infection had not completely disappeared in man. An analysis was made of all cases of pulmonary tuberculosis from 1944 to 1955. The annual incidence was 100 cases in 1948, 50 in 1952, and 27 in 1955. The bovine tubercle bacillus was isolated from 3 to 6 cases in the earlier years, and 2 to 3 in the later years, and the author after taking such factors as age and occupation into consideration, concluded that the later cases had arisen from what were, for a long time, latent infections.

Smith (20) referred to the previous investigations of Wilson, Blacklock & Reilly (21) who found that in England, in 1943–45, 28% of the reported cases of tuberculous meningitis and 25·7% of cases of surgical tuberculosis were of bovine origin, while in Scotland the incidence was 11·1 and 33·8% respectively. The author discussed the three methods available for control, namely, pasteurization, examination of samples of milk for tubercle bacilli, and government action to ensure safe milk. He stated that in October 1957, 66% of all cattle were in attested herds in England, while the figures for Wales were 92% and for Scotland 90%. It is expected that by the early 1960's bovine tuberculosis will be a thing of the past.

In 1957 there appeared in the *Zeitschrift für Arztl. Fortbildung* a series of papers on tuberculosis of animals and man. In two papers Laugwitz (22,23) discussed tuberculosis in animal husbandry and animal sources of tuberculosis infection for man. The author investigated 100 patients

suffering from tuberculosis who were associated with animal husbandry. In 31 of the patients the typing of the tubercle bacillus was successful and 7 were found to have infections due to the bovine type. Five of the patients suffered from pulmonary tuberculosis and 2 from infections of bones. In the second paper, the author discussed the mode of infection of man from various animal sources and concluded that man's greatest danger is cattle, but goats, cats, dogs, horses and wild animals kept in captivity in zoological gardens also present a hazard. From adult patients, Krebs(24) isolated 8 bovine and 1222 human strains of tubercle bacilli from sputum and other pulmonary specimens and 6 bovine and 70 human strains from non-pulmonary materials. From children, 6 bovine strains were obtained from 38 specimens of cerebro-spinal fluid, 6 from 9 neck glands, 2 from the lungs, 1 from a mesenteric gland and 1 from a discharging ear. Comparative cultural tests showed that the Santon egg-yolk medium without glycerine was superior to Holn and Kirchner's medium with glycerine for the isolation of tubercle bacilli of the bovine species. Schreiter(25) discussed the epidemiological and clinical findings in children with tuberculosis due to the bovine type of bacillus. Of 108 strains isolated from children, 15 proved to be of bovine type. Investigation showed that the source of infection was not known in 8, a tuberculous cow was suspected in 2, and tuberculosis was present in other members of the family in 4 instances. Goerttler(26) discussed the risks incurred by man in handling tuberculous cattle. In addition to infection being derived from alimentary, respiratory and skin lesions, he mentioned the possibility of infection occurring through the accidental injection while employing syringes used for the tuberculin testing of animals. Finally, Bollbrecht(27) discussed possible sources of infection in small domestic animals. He pointed out that monkeys and dogs are both susceptible to the human and bovine types, but cats are more susceptible to bovine than human infection. Rodents have been found infected with the human type but birds are mainly susceptible to the avian type, and only in some degree to human and bovine strains.

Viallier(28), in the Lyons area of France, isolated 311 strains of tubercle bacilli. Twelve strains of bovine tubercle bacilli were obtained and all came from materials obtained from children, being strains from cervical lymph glands.

From 1949 to 1952, twenty-two Public Health Laboratories in England and Wales collaborated in an investigation(29) into the presence of tubercle bacilli in milk which had been subjected to pasteurization by the holder method or by the H.T.S.T. method. At the same time, the phosphatase test was applied to all samples and the results were compared with the results of guinea-pig inoculation for tubercle bacilli. Davis(30,31) had carried out a similar investigation, and in Table 2 his results are compared with those obtained by the Public Health Laboratory Service.

Table 2. *Pasteurized milk examined in different parts of England and Wales by the phosphatase test and by the guinea-pig test for tubercle bacilli*

(Results obtained by Davis 1953–55 and the Public Health Laboratory Service (P.H.L.S.) 1949–52 compared.)

		Holder method		H.T.S.T. method	
		T.B. positive	T.B. negative	T.B. positive	T.B. negative
Phosphatase positive (failed)	Davis	4	236	0	100
	P.H.L.S.	8	193	2	91
Phosphatase negative (passed)	Davis	2	3632	2	6665
	P.H.L.S.	0	524	2	3505
Totals	Davis	6	3868	2	6765
	P.H.L.S.	8	717	4	3596

The findings of the Public Health investigators were summarized as follows: (1) Of milk treated by the holder method, 8 out of 201 giving a positive phosphatase reaction produced tuberculosis on injection into guinea-pigs; none out of 524 giving a negative phosphatase reaction did so. (2) Of milk treated by the H.T.S.T. method, 2 out of 93 phosphatase-positive samples gave rise to tuberculosis in guinea-pigs but there was no reason to believe that these two were raw milks

sampled in error. Of 3507 phosphatase-negative samples, one was known to contain tubercle bacilli, but this was apparently derived from a filling machine used for tuberculous raw milk. (3) Since only a small proportion of phosphatase-negative milks were injected into guinea-pigs, the figures recorded could not be used for calculating the real incidence of tubercle bacilli in pasteurized milk. They indicated, however, the efficiency of the holder and H.T.S.T. methods since 8 out of 725 (1·1%) holder-treated and 1 out of 3600 (0·03%) H.T.S.T.-treated milks contained tubercle bacilli. (4) It was concluded that the H.T.S.T. method of pasteurization was more effective than the holder method, and that the results indicated the necessity for more stringent control of processing.

Eissa, El-Sadek & Shadi (32) in Egypt examined individual samples of milk for tubercle bacilli from 45 cows and 42 buffaloes by guinea-pig inoculation. Two samples from each group were positive.

SALMONELLA INFECTIONS

(*a*) *Typhoid and paratyphoid fevers*

No report of an outbreak of typhoid fever due to the contamination of milk has been encountered in recent literature. An outbreak of *Salmonella paratyphi* B was mentioned in the last review, and more details are now available. O'Donell & Schrack (33) described the outbreak which occurred in Lancaster County, Pennsylvania, in December 1955. No less than 279 individuals were involved, and the epidemiological investigation incriminated milk as the vehicle, the source being traced to a permanent carrier employed by a local dairy. The patients ranged in age from 5 months to 65 years, but 80% of the patients were less than 16 years old. Fever, anorexia, diarrhoea, headache, rose spots, vomiting, and abdominal pain or tenderness were the most frequent symptoms. Stool cultures were made from all patients and *S. paratyphi* B was found in the stools of 208. Blood cultures were made in 46 instances and proved positive in 35; agglutination tests were positive in 105 of 128 patients. Of the 71 patients with negative stool cultures, 1 had a positive blood culture and 31 had positive agglutination tests. Thus, positive laboratory evidence was obtained in 240 patients. The 59 patients who were hospitalized were treated with chloramphenicol in varying doses. Less than 1% of the 279 patients, 80% of whom had received chloramphenicol, were excreting the organism at the end of 6 months, and all patients had a bacteriologic recovery at the end of 9 months. Chloramphenicol therapy may have been of value in reducing the convalescent carrier rate.

(*b*) *Food poisoning*

Buxton (34) has published a review, *Salmonellosis in Animals*. This book contains chapters on the general characteristics and classification of salmonellae, the distribution of serotypes, the symptoms and lesions of infection, the epidemiology and immunology, antibacterial substances, and public health aspects of such infections. A detailed account is given of the relationship of the various types to infections in cattle, sheep and goats, and Table 3 has been compiled to summarize this. It is noted that in bovines *S. dublin* is the most commonly encountered type, though in some areas *S. typhi murium* occurred with equal frequency, and no less than 28 different types were isolated at one time or another. In sheep and goats *S. abortus-ovis* and *S. typhi murium* are the predominating types, though 17 types in all are recorded as occurring in these animals. The author points out that the common source of milk infection is the faeces from a carrier animal, and only occasionally infection may be primarily in the milk of animals acutely infected with the disease. A precaution, therefore, of the greatest value is the pasteurization of milk, which not only renders the milk free from *Salmonella* infections, but ensures that milk products such as butter and cheese will not be contaminated. Finally, the volume contains a very extensive bibliography.

The incidence of *Salmonella* infection in calves in Denmark has been studied by Müller (35). Between July 1948 and April 1956, 8016 dead calves were investigated bacteriologically, and 2·17% were found to harbour salmonellae, the two main types being *S. dublin* (2·17%) and

S. typhi murium (0·26%). Other types isolated were *S. enteritidis* (2), *S. enteritidis* var. *dansyz* (3), *S. bredeney* (2) and one each of *S. dusseldorf*, *S. saint-paul* and *S. chester*.

The Annual Report on Animal Health Services in Great Britain for 1955 (36) states that up to 1955 the incidence of salmonellosis in cattle was very low, but in 1955 a considerable increase in the number of such infections occurred, particularly in South Wales. Discussing the marked increase of food poisoning in Great Britain, Savage (37) pointed out that no outbreak can be fully explained unless four factors are solved. These are: (1) the agent responsible for the symptoms, (2) the vector responsible for conveying the causal agent to man, (3) the reservoir from which the infecting organism originated, and (4) the path from the reservoir to the infected vector.

Table 3. Salmonella *serotypes which have been isolated from cattle and from sheep and goats*

Cattle		
abony	*kentucky*	*panama*
abortus ovis	*minnesota*	*paratyphi* B
anatum	*moscow*	*pullorum*
bovis morbificans	*new brunswick*	*rostock*
brancaster	*newington*	*tel-aviv*
cholerae suis	*newport*	*thompson*
derby	*oranienburg*	*typhi murium*
dublin	*oregan*	*vejle*
gallinarum	*orientalis*	*worthington*
give		
Sheep and goats		
abortus ovis	*dublin*	*onderstepoort*
anatum	*enteritidis*	*paratyphi* B
bovis morbificans	*essen*	*poona*
bredeney	*meleagridis*	*potsdam*
cholerae suis	*new brunswick*	*typhi murium*
derby	*newport*	

Rasch & Richter (38) described an outbreak of salmonellosis due to *S. heidelberg* in farm workers and cattle. A cow was sent to an abattoir for emergency slaughter, and the organism was isolated from various organs. Later, 6 people working on the farm were all found to be excreting the same organism and so also were 12 out of 29 cattle. *S. heidelberg* was also recovered from water in the pastures and from an adjacent stream. One of the infected cows continued to excrete the organism in the faeces for 3 months. The slaughter of this animal and the application of hygienic measures removed the infection, the original source of which was believed to have been fish meal.

The reports prepared by the Public Health Laboratory Service on food poisoning in Great Britain during 1955 and 1956 (39, 40) showed that comparatively few incidents were actually due to milk in the fresh or canned form, and the relevant particulars are given in Table 4. In 1955 an outbreak of *Salmonella* infection was associated with liquid milk. Then, 17 out of 37 individuals became infected with *S. typhi murium* and showed symptoms of illness after drinking milk bottled subsequent to pasteurization. The contamination was thought to be due to cardboard caps infected with mouse faeces being placed on the bottles by hand. In the second incident, 6 out of 8 members of a family became ill after eating junket made from cow's milk. In 1956 two incidents occurred. In the first, due to raw milk infected with *S. typhi murium*, 3 primary and 3 secondary cases occurred. In the second, 4 individuals developed food poisoning in a children's ward, and here the vehicle of infection appeared to be dried milk which had become infected after the can had been opened, the organism causing the infection being *S. newington*. Wright, Norval & Orr (41) reported two outbreaks of food poisoning due to *S. thompson*, one being due to the use of infected Chinese egg albumen in a bakery and the other to a cow with a septicaemic type of disease producing infected milk. A medical practitioner in an outlying district of Edinburgh notified the Medical Officer of Health that his own 9-month-old child had an acute *Salmonella* infection, and that his elder child was also ill with gastro-enteritis. He strongly suspected that the milk supply, which came from a nearby farm, was the source of infection. The farm which produced tuberculin-

tested unpasteurized milk was immediately visited, and it was found that the byreman was also ill and, later, the bacteriological investigation showed 5 more symptomless excreters, 3 being relatives of the byreman and the other 2 being the manager and the vanman. It was found also, that five of the customers consuming the milk had symptoms of food poisoning. The veterinary inspection of the cattle showed that one cow was acutely ill. She was sent for immediate slaughter and specimens of faeces and milk from this animal showed *S. thompson*: the actual source of the cow's infection was not determined. Boyd (42) described an outbreak of salmonellosis in Fifeshire. The Divisional Veterinary Officer advised the Health Department that the illness in a cow on a farm was due to *S. newport*. The herd consisted of 48 attested milk cows and followers and only one animal appeared to be ill, though later a newly-born calf also developed the disease and died. Twelve clinical cases among the staff on the farm were recorded and 5 members of the general public who had consumed the infected milk developed clinical symptoms. The cow recovered from the acute infection but as she continued to excrete *S. newport* she was slaughtered.

Table 4. *Food poisoning of all types*

(Vehicles of infection, presumed causal agents and numbers of general plus family outbreaks when cause was traced to a specific food.)

	1955			1956		
		Milk			Milk	
	All foods	Fresh	Canned	All foods	Fresh	Canned
Salmonella typhi murium	27	1	—	20	1	—
Other salmonellae	12	—	—	11	—	1
Staphylococci	84	1	1	64	1	—
Clostridium welchi	80	—	—	60	—	—
Clostridium botulinum	1	—	—	0	—	—
Other organisms	7	—	—	13	—	—
Clinical	4	—	—	1	—	—
Not discovered	97	—	—	99	1	—
All agents	312	2	1	268	3	1

All incidents of food poisoning (general outbreaks, family outbreaks, and sporadic cases) including those not traced to specific foods: 1955, 8961; 1956, 7713.

Dysentry

In the publications reviewed only one report of an outbreak of dysentery due to infected milk was recorded. In September 1955 (43), in a small South African town, 64 out of a total of 125 children and 9 adults at a particular school were affected, while in addition some 26 individuals in the town generally also suffered. The school outbreak occurred mainly among non-European children aged 3–8 years, and no less than 3 non-European children and 1 European child died. The outbreak was explosive and appeared to have an incubation period of 7 hr. The symptoms were vomiting, headache, abdominal cramps with tenesmus, and often blood, diarrhoea with, in many cases, pyrexia. The vehicle of infection appeared to be a specially prepared type of sour milk made with *Streptococcus cremoris* as a starter. Fresh milk in 18-gallon quantities was treated with *Str. cremoris*, and after souring was boiled and left to cool in the containers with the lids off. It was at this stage in the processing that the material was apparently infected. Bacteriological examination showed that 6 non-European employees in the dairy were infected with the *newcastle* dysentery bacillus (*Shigella flexneri* type 6) and the same organism was obtained from 1 fatal case and many of those affected. The occurrence was characteristic of most milk-borne dysentery outbreaks due to infected milk.

STAPHYLOCOCCAL INFECTIONS

The importance of *Staphylococcus aureus* in cross-infections in surgical and other wards of hospitals, in food poisoning in human beings, and in infections of animals, is evidenced by the increasing attention being paid to this organism. Its capacity to develop resistance to penicillin

has now made it a much more dangerous pathogen than the haemolytic streptococcus. Thatcher & Simon (44) have examined some physiological and toxigenic properties of the genus *Micrococcus* in relation to taxonomy. The general conclusions reached by these workers were that: (1) postulation of the two genera *Micrococcus* and *Staphylococcus* was not warranted on a taxonomic basis; (2) variation in pigment production, being on occasion reversible offers a dubious basis for distinction between varieties; (3) to separate pathogens from non-pathogens, standard phages are the best means of recognition since the action of these phages is limited to coagulase positive strains; and (4) micrococci are a group of strains of bacteria which possess many overlapping characteristics, making their differentiation unusually arbitrary.

A further paper by Thatcher & Simon (45) presents a comparative appraisal of the properties of 'staphylococci' from clinical sites and from dairy products. In the author's words the study was undertaken in order to establish for each of the two groups of organisms: (*a*) species relationship according to Bergey; (*b*) the production of specific toxins by individual isolates; (*c*) the production of coagulase and phosphatase and the relationship of these properties to the ability of specific isolates to produce toxins; (*d*) the comparative ability of members of the various phage groups to produce coagulase, phosphatase and toxins; and (*e*) to what degree staphylococci isolated from cheese or butter may contribute to infections in man, as revealed by the recurrence of phage types common in these foods, among hospitalized human patients. The conclusions reached after this extensive investigation are of great interest to those working on this aspect of bacteriology and can only be summarized here. Mannitol-positive staphylococci isolated from dairy products and from clinical sources showed comparable production of coagulase, phosphatase, alpha- and beta-haemolysins, and enterotoxin. *Aureus* and *albus* strains occurred in roughly equal proportions from human sources, but *aureus* strains from dairy products were more consistent producers of alpha- and beta-haemolysins; all strains randomly selected from dairy products, were lysed by phage type 42 D and were, therefore, members of phage group IV considered to be of bovine origin. Representatives of this group were not found among 139 strains from human sources, and this suggested that staphylococci in butter and cheese were of minor significance as a cause of infection in man except through food poisoning. Coagulase and phosphatase production were incomparable in relation to toxin production, though coagulase was the more dependable indicator for the production of at least one of the three toxins. Certain antibiotic resistant strains from clinical sources were originally coagulase negative, but after 5 months of routine transfer were shown to be coagulase positive members of phage group III.

An annotation in the *British Medical Journal* (46) discusses penicillin-resistant staphylococci in milk, with particular reference to the papers of Gould (47), Tee (48), Steeds & Smith (49) and Smith (50). It is suggested that the wholesale use of penicillin in the treatment of mastitis in cows is a contributory factor in the production of penicillin-resistant strains, and is a matter of concern to both the medical and veterinary professions.

Tee (48) carried out an investigation into the incidence of penicillin-resistant *Staphylococcus aureus* in random samples of milk from dairy herds in Dorset County. Some 120 herd samples out of a total of some 3000 were examined in 1954 and again in 1955. Penicillin resistant *Staph. aureus* strains were found in 11 or 9% of samples in 1954, and in 47 or 37% of samples in 1955. Sufficient information was forthcoming to show that the widespread use of penicillin in the treatment of staphylococcal mastitis was probably responsible for the emergence of penicillin resistant strains.

Research, partly explaining why milk, raw or pasteurized, is not a frequent cause of staphylococcal food poisoning has been done by several workers. Thus Smith (50) carried out extensive investigations into the multiplication of *Staph. aureus* in cow's milk, and he suggested that (1) within the temperature range at which milk is usually kept there is comparatively little multiplication, and (2) that the presence of other bacteria in milk seems to limit the multiplication of staphylococci, although no proof was obtained that anti-staphylococcal substances were present. It seemed probable, therefore, that food poisoning would be more likely to arise when milk production was carried out under good hygienic conditions than in circumstances causing

considerable bacterial contamination. Bacterial counts following the addition of staphylococci to raw milk and to milk which had been previously heated at 100° C. for 5 min. showed smaller counts in the raw milk samples. This was apparently due to a heat-labile clumping factor being present in the raw and not in the heated milk. Its effect could be overcome by strong agitation of the diluting fluid when the counts were made. Again Jones, King, Fennell & Stone (51) reported on a series of examinations and experiments carried out in four Public Health Laboratories. *Staph. aureus* was fairly common in certified and other forms of milk, and the strains from milk showed no difference from known pathogenic strains. At temperatures of 30 and 37° C. there was a considerable multiplication with little difference between the two temperatures. Streptococci which produced lactic acid tend to inhibit growth of staphylococci at lower temperatures. The authors state that staphylococcal food poisoning due to milk is unlikely to occur unless the contamination is due to an infected udder and the milk is maintained at a fairly high temperature. Suitable conditions for toxin production are therefore, on the whole, unlikely, and this would account for the rarity of milk-spread *Staphylococcus* food poisoning.

Worseck (52) examined 45 strains of staphylococci from cases of bovine mastitis. In broth at room temperature some 15 strains were capable of producing toxin within 2 days. Some strains were very resistant to heat, and experiments carried out on kittens showed that the toxins obtained from 16 strains could produce diarrhoea. Barnum & Fuller (53) used phage typing methods to identify 591 strains of haemolytic staphylococci isolated from cases of mastitis and from the normal quarters in cows. Slightly over 81% of the strains could be classified into 5 groups. The Weybridge Laboratory (54) dairy herd is now being used to study the epidemiology of mastitis due to staphylococci. No less than 2386 strains of staphylococci were isolated and phage typed during the year, and three mastitis-producing strains were frequently isolated from the body surfaces of the cows and from the milkers' hands. New strains are being introduced experimentally into the herd and the spread of infection is being studied.

In the food poisoning reports of the Public Health Laboratory Service for 1955 and 1956 (55,56) several instances of infection are recorded. In one outbreak 6 out of 8 persons in a family became ill 4 hr. after eating junket made from cow's milk. The strain of *Staph. aureus* isolated from the junket was found to have the same phage type as that obtained from the original milk. In another incident due to canned milk *Staph. aureus* was isolated from the patients and from the can which had been opened some 3 days prior to being used. The third recorded incident occurred in a hospital ward and was associated with unpasteurized milk, while in the fourth outbreak 19 school children were affected after drinking pasteurized milk. Hammer (57) records the occurrence of gastro-enteritis in 5 people who had consumed goat's milk. Examination of the goat showed that it had a mastitis due to *Staph. aureus*.

The work of Anderson & Stone (58) directed more attention to dried milk as a possible source of staphylococcal food poisoning. A paper by Hobbs (59) states, as a result of the examination of numerous samples of dried milk, that a method of producing the substance free from coagulase positive staphylococci had not so far been evolved. The possible sources of contamination of dried milk were: (1) infected milk and milkers and the multiplication of the organisms during storage; (2) contamination of the plant by milk or from milk via personnel; and (3) air-borne or human-borne contamination of the milk in the seal-pot or balance tank. Again, Hawley & Benjamin (60) noted that the bacteriological problems which arise in the preparation of dried milk are largely due to the size and complexity of the plant, to mechanical breakdowns, and to failure of the human element. They discussed such aspects of production as general effect of plant hygiene, vacuum concentration, seal-pot and balance tank, atomizer feed tank, the effect of contamination, spray-drying, packaging, and prevention of contamination. Finally, in their conclusions they make suggestions for pre-heating and for the improvement of the plant in order to eliminate reservoirs where multiplication of organisms could take place. Crosby & Mystil (61) have also examined the influence of manufacturing processes on the staphylococcal content of dried milk. The heat employed in vacuum evaporation reduced the numbers of *Staph. aureus*. One strain was found to be capable of multiplication at evaporating temperatures up to about 43° C. Above

49° C. this organism's activity declined rapidly and at 56° C. was eliminated. A recent paper illustrates the problems which arise from contaminated dried milk. Armigo, Henderson, Timothee & Robinson (62) reported that in Puerto Rico, between 23 January and 21 February 1956, 19 separate outbreaks of gastro-enteritis occurred in children who had lunch at school. These outbreaks were reported from 16 schools, 3 having experienced two such episodes. In all 775 children of the 4094 eating at these lunch-rooms were ill. The symptoms were characteristic of staphylococcal food poisoning with a short incubation period, nausea, vomiting, and diarrhoea without fever. Recovery was complete within 24 hr. The epidemiological evidence predicted that spray-dried milk powder was the most likely source. Reconstituted dried skim-milk powder was used extensively throughout the island and was issued from various warehouses. All outbreaks, with two exceptions, were confined to the schools supplied by the warehouse at the port of San Juan. Over 90 samples of 17 foodstuffs were examined by the Commonwealth Health Laboratories with negative bacteriological and toxocological results. On 14 February, however, a can of dried milk was obtained from one school at which, on the previous day, an outbreak had occurred. Some of the milk was reconstituted and one of the authors (R. A.) consumed a glassful, typical symptoms of staphylococcal food poisoning occurring 2 hr. later. Further experiments were then undertaken utilizing 26 adult volunteers. Thirteen of these were given a glass of reconstituted milk obtained from the school, and the other 13 partook of a glassful of milk made from material purchased from a commercial source. Nine of those who consumed the reconstituted school milk developed symptoms, 5 being moderately ill and 4 mildly. Two individuals, who partook of milk commercially obtained, also showed mild symptoms. Spray-dried milk solids from the United States have been used in the Puerto Rican government programmes for over 10 years without previous reported trouble, and at the present time some four million pounds are used annually. At the time of the outbreaks no less than eight shipments of milk powder were in the island warehouses and only one of these was intact. Two shipments comprising 963,000 pounds in twenty different lots and processed by seven different companies came under suspicion. After an intricate investigation it was shown that only two out of the twenty lots were represented in the stocks of every school experiencing an outbreak, and another one lot was stored at one school only. Further investigation showed that all three suspected lots had been prepared by one producer. Although the bacteriological studies were negative, the authors believe that the clinical symptoms were characteristic of food poisoning due to staphylococcal enterotoxin which is more resistant to heat than the organism.

The question of cheese as a possible source of food-poisoning has been examined by Thatcher, Simon & Walters (63). Their paper summarizes the results of studies made to determine: (1) incidence in Canadian cheese, and in milk supplied to cheese factories, of extraneous matter and of specific bacteria usually considered to be of public health significance; (2) the comparative sanitation of cheese factories; (3) presence in marketed cheese of specific bacterial toxins; (4) the extent to which cheese may serve as a vehicle for the distribution of pathogens. Their work disclosed considerable contamination, indicating faulty methods of milk production. A substantial part of the cheese made from pasteurized milk also showed that contamination had occurred after pasteurization. Experience suggested that the proportion of cheese which would allow the development of a clinically significant amount of enterotoxin is very small, though under appropriate unknown conditions it can occur.

BRUCELLOSIS

Spink (64) has published a new volume on the nature of brucellosis, which the author describes as the biography of a disease, and the autobiography of a laboratory and a clinic devoted to a study of that disease. The first chapter is devoted to the evolution of the concept that brucellosis is a disease of animals and man, and the second to the brucella cell; the third chapter describes the reservoir and consequently discusses the infection in goats, sheep, cattle, and other domestic animals; the fourth deals with the dissemination of brucella organisms, and numerous events associated with the distribution of *Brucella melitensis* through goat's milk and fresh goat's cheese

are described. It is noted that the ingestion of ripened cheese carried no hazard. As regards cow's milk, the remarkable features emerging from the epidemiological studies on *Br. abortus* were the high incidence of bovine brucellosis in the United States, the large number of persons with serological evidence of having been infected through the ingestion of cow's milk, and the relatively small number of cases of illness. No cases of human brucellosis due to *Br. abortus* have been traced to cheese, ice cream, butter, or contaminated water. Details are given of epidemiological studies in relation to various conditions and employments. Further chapters are devoted to the pathogenesis, course, complications, diagnosis, and therapy of brucellosis. The prevention of human brucellosis and the elimination of the disease in animals, the author explains, constitute two separate problems, and the resolution of the first is dependent on the second. In this connexion pasteurization of milk, the elimination of brucellosis in animals, the immunization of animals, and eradication programmes are fully discussed. In the last chapter the various problems yet to be solved in connexion with the infection are discussed.

The Annual Report on Animal Health Services(65) states that during 1955 samples of milk from two farms in east and west Sussex were found to contain an organism having characteristics indistinguishable from *Br. melitensis*, and the infected animals were dealt with under the Brucellosis Melitensis Order of 1940. During 1956(66) twenty-three cattle on 4 farms were slaughtered on account of the excretion of *Br. melitensis*. All told, since 1940, *Br. melitensis* has been isolated from cattle on 42 farms in different parts of the country, usually from milk, but in a number of cases from the foetuses of cows which had aborted. The report goes on to state, however, that at no time has this organism been associated with recognizable illness in humans, and it appears, therefore, that at worst it is no more pathogenic for humans than *Br. abortus*. In the section(67) on the laboratory work carried out at the Central Veterinary Laboratory, Weybridge, an analysis has been made of the results obtained in the typing of brucella strains at Weybridge since 1953. *Br. melitensis* has been detected in 27 herds, and 17 herds have been found to be infected with *Br. abortus/melitensis*. The latter are strains which are like *Br. abortus* in cultural and biochemical characters and like *Br. melitensis* serologically. *Br. suis* and *Br. melitensis/abortus*, i.e. *melitensis* biochemically and *abortus* serologically as found in certain other countries, have not been recovered. The laboratory report states that issue of International and National Standard *Brucella* agglutination sera, monospecific typing sera, and type strains of *Brucella* were continued. Dalrymple-Champneys(68) expresed the view that the *melitensis* strains isolated from bovines in Great Britain were devoid of virulence for humans. In an attempt to obtain some satisfactory explanation for this problem Cruickshank(69) carried out a study of the duration of bacteraemia after the inoculation of various strains into guinea-pigs. Strains of *Br. abortus* and *Br. melitensis* obtained from bovine sources in Great Britain were inoculated into guinea-pigs, and samples of blood were collected at weekly intervals by cardial puncture and cultured. It was found that strains of low virulence were almost always cleared from the blood within a week, whereas virulent strains produced a bacteraemia for 4 weeks or longer. Four strains of *Br. melitensis* isolated from cows behaved in the same way as the typical and virulent strains of *Br. abortus*, and produced a prolonged bacteraemia. The author comments on the fact that no human infections (apart from laboratory accidents) have been reported as arising in Great Britain, and suggested that the absence of such cases might be due to: (1) the small number of herd milks containing *Br. melitensis* and (2) the difficulty of recovering the organism from human cases, so that an occasional human case might occur and not be recognized. It would seem that the problem of the relatively low virulence of *Br. melitensis* from bovine sources for man in this country requires much further investigation.

Hendricks(70) has described the epidemiological features of brucellosis in Iowa. Physicians reported 720 cases during 1952–53, and of these 614 were males and 106 females, the majority being in the groups aged 20–39 years. The infecting organisms were *Br. suis* 61%, *Br. abortus* 26% and *Br. melitensis* 13%. About 80% of the patients had a history of occupational or other contact with farm animals. Swine were the main reservoir of infection but as most of the farming people drank unpasteurized milk there was a considerable number of cases due to *Br. abortus*.

Wilson (71) reported a steady rise in the incidence of brucellosis since 1947 in Victoria, Australia. From 1950 to 1955 inclusive, official reports in all states showed that more than half the cases occurring in Australia were in Victoria, and that in all states except South Australia fewer cases were reported from the capital than from the rest of the State. In Victoria, the organism concerned was the bovine species *Br. abortus*. There were no accurate figures on the incidence of contagious abortion in cattle, but there was no doubt that large quantities of infected milk were still being drunk, particularly in rural areas. Few infections were contracted in this way, and the preponderant incidence of brucellosis was among those who had direct manual contact with cattle. The attenuated strain 19 could produce clinical brucellosis in man. The prevalence of brucellosis in East Africa was reviewed in the *Lancet* (72). It was noted that in Uganda the largest number of cases was found in the predominantly cattle country in the western region near Lake Edward, and infection was mostly due to *Br. melitensis*. In Kenya the number reported had risen from 25 in 1945 to 238 in 1950 and more than a 100 each year since then. In the African population the infection was almost entirely due to *Br. melitensis* but the few cases amongst Europeans were usually due to *Br. abortus*. Furthermore, Wright, Cook & D'Souza (73) have noted an increased incidence of infection in middle-aged and elderly Africans. Polding (74) of the Veterinary Department of Makerere College has put forward the view that rainfall encourages the spread of the disease in animals, by aggregation, by increasing susceptibility and by longer survival of the organisms on wet surfaces. Lawy (75) examined during the period 1948–50, in the Lincolnshire area of England, 6168 human sera submitted for the Wassermann reaction, and a further series of 2023 sera during the period 1950–53. During the first period 1·05% of sera showed agglutinins for *Br. abortus* to a titre of 1/80 or more, whereas during the second period only 0·64% gave a positive test. The author believes that this fall in the percentage positive may represent a genuine fall in the proportion of infected persons and may be associated with: (1) an increase in the quantity of milk subjected to heat treatment, (2) a decrease in the number of producer retailers of raw milk, and (3) a lessening of infection in cattle due to immunization. For screening purposes the Huddleson's slide-agglutination test was found to be more sensitive than the tube method. Nussbaum (76) made a study of the epidemiology of undulant fever in Switzerland during the period July 1953 to June 1956. In that period 167 new infections were notified for the whole of Switzerland and of these 15 were from the canton of Berne. Further inquiries, however, in this canton brought to light a total of 54 new infections, and the canton Veterinary Officer said that during the period no less than 388 cattle had been found to be excretors of *Br. abortus*. The incidence of the disease was about 1 per 10,000 of the population in the whole area. In farmers, however, in close contact with infected animals, the percentage incidence was 2, which is 200 times higher than the over-all figure. The disease was extremely rare in the City of Berne, only one case being reported. This low incidence was attributed to pasteurization of milk, cream and butter. Suggestions are made for extension of pasteurization and the complete investigation of all sources in cases occurring in rural areas.

A considerable amount of work has been done on the comparative value of different tests for the detection of brucellae in milk and milk products. Sironi (77) examined milk samples from 578 cows serologically positive and subjected them to biological tests in guinea-pigs and to cultural tests on a medium containing penicillin and on a medium containing actidione and bacitracin. The penicillin medium was unsatisfactory owing to exuberant growth of fungi, but better results, comparable with those of biological tests, were obtained with the other medium in which actidione inhibited fungi. In view of the sensitivity of some brucella strains to bacitracin it was considered that a medium containing actidione, as fungus inhibitor, and gentian violet, as bacterial inhibitor, would be more suitable. Vanini & Neri (78) carried out comparative tests on the value of various media for the recovery of *Br. melitensis* and *Br. abortus* from artificially infected milk. After centrifuging, the mixed cream and sediment were sown on media which included malachite green, various kinds of violet, penicillin, polymyxin B and bacitracin. After many experiments the following medium was found to be very satisfactory: tryptose agar 1000 ml., actidione 100 mg., polymyxin sulphate B, 4000 units, bacitracin 15,000 units, ethyl violet (aqueous

solution 1/8000) 10 ml. Colonies on this medium appeared, on incubation at 37° C., in 36 hr. After these preliminary experiments the medium was used for the detection of *Br. abortus* and *Br. melitensis* in individual cow's milk. Cultures were grown at 37° C. with and without CO_2, and again the results suggested the medium was highly selective and of considerable practical value. Carrière, Roux & Suire(79) have described a medium suitable for the isolation of brucellae from dung, milk, and aborted material, the object being a medium in which contaminants such as Gram-positive cocci, *Escherichia coli*, *Pseudomonas*, *B. subtilis* and *Proteus* are reduced though not of necessity eliminated. Strains of *Br. melitensis*, *abortus* and *suis* were tested for their resistance to antibiotics and other similar substances. The basic medium was Albimi-agar with 2% glycerine and adjusted to a pH of 6·8. This was sterilized and before use the following was added per litre: a watery solution of sunoxol (a neutral sulphate of oxyquinoline) to a final concentration of 1 in 320,000, eosin to a final concentration of 1:40,000, 30 mg. of nystatin in alcoholic solution 900 mg. polymyxin with 30,000 units bacitracin. All three types of *Brucella* were sensitive to certain concentrations of neomycin and framycetin and *Br. melitensis* displayed some sensitivity to penicillin. These substances were, therefore, not included in the medium.

A working Party of the Public Health Laboratory Service(80) investigated the value of the milk ring test (M.R.T.) and related tests with the purpose of determining: (*a*) their value as evidence of the presence of *Br. abortus* in herd milk; (*b*) the value in the indentification of herds infected with *Br. abortus*; and (*c*) the effect of vaccination with strain 19 vaccine on the interpretation of the several tests. Comparison of the M.R.T. and the biological test in eight laboratories showed that out of a total of 22,346 samples 46·43% gave a positive M.R.T. and 11·29% gave a positive biological test. Positive results with both M.R.T. and biological tests were obtained in 23·27%, and 0·91% gave a positive biological test and a negative M.R.T. Furthermore, there was an association between the intensity of the ring test reading and the excretion of brucella organisms, the greater the intensity of the reaction, the higher was the percentage of positive biological results. A comparison of the ring test with the whey test, the plate test, and the capillary tube test, was also carried out. It was found that, with herd milks, the proportion of samples reacting negatively by one of the agglutination tests but yielding a positive biological result was less in the ring test (0·4–0·8%) than in the whey test (3·1%), the plate test (2·2%) or the capillary tube test (1·6%). Similarly, the proportion of samples giving a doubtful result which was positive on biological tests was smaller in the ring test (0·4–0·8%) than in the plate test (17·7%) or the capillary tube test (9·6%). Based on these standards the ring test appeared to be the most suitable for use as a screen test, on account of both its simplicity and the low proportion of false negative reactions. Sole reliance cannot be placed on any of the milk-agglutination tests for the detection of *Br. abortus* in milk since in only 60–75% of samples which reacted positively, *Br. abortus* could be demonstrated by guinea-pig inoculation. Full details are given of the techniques of the various tests employed. Jameson(81) carried out research on the value of the capillary test, the ring test, and cultural methods in the detection of *Br. abortus* in infected quarter-sample milks. The ring test was first devised by King(82) and its value has already been assessed by the authors of the previously summarized report. Jameson found that in a series of 2959 samples of milk, 173 positive results were obtained by culture and of these 171 gave strong positive results and 2 gave a positive reaction with the capillary test. The ring test proved less satisfactory for screening quarter samples and it is suggested that this was due to the occurrence of occasional samples with a low fat content. The same author(83) devised a cup test for the rapid serological screening of guinea-pigs infected with *Br. abortus*.

The guinea-pigs were bled 6 weeks after being inoculated with milk. Each serum was diluted 1/2½ and 1/25 and a volume of each dilution was placed in the depressions of a specially prepared Perspex plate. Then a volume of Weybridge Standard *Br. abortus* concentrate diluted 1/10 was added. The mixtures were then incubated at 37° C. for 1 hr. and left on the bench for 15 min. before being read. Out of a total of 7000 tests, 5972 gave negative results. The sera of 1034 guinea-pigs were positive by the cup test and all except 9 were positive by tube agglutination. *Br. abortus* was isolated from the spleen of 5 of these 9 animals and from 886 of the 1025 animals

positive both by the cup test and by tube agglutination. Spleen cultures were unsuccessful in 143 guinea-pigs positive by the cup test and in 139 positive by tube agglutination. The screening technique was more sensitive than tube agglutination, though, for practical purposes, it was equally specific. Fourteen per cent of spleen cultures of guinea-pigs positive by either test were unsuccessful, and the probability of isolating *Br. abortus* from the spleen of an inoculated animal increased progressively with the titre of brucella agglutinins reached in the serum after 6 weeks.

The survival of *Br. abortus* and *Br. melitensis* in butter has been studied by Gargani & Guerra (84), who carried out experiments using two standard strains to infect milk. From the infected milk cream was obtained and butter made and samples were taken at various times up to 8 weeks and inoculated into guinea-pigs. After 15 days, and thereafter at intervals of 7 days, blood was obtained from the inoculated animals for agglutination test. When the titre of agglutinin in any of the sera reached 1/640 to 1/1280 the animals were killed and spleen and other organs were taken for culture. The results indicated that at doses of organisms from about 10^5 per ml. upwards there was no difference in survival times between the 2 strains but in lower concentrations *Br. abortus* survived longer. The development of acidity in butter was not harmful. The authors suggested that when other workers have found short periods of survival in butter this has probably been due to the presence of relatively small numbers of brucellae. They conclude that it is essential to pasteurize all milk to be used in the preparation of butter.

LEPTOSPIROSIS

Alston & Broom (85) have published a book giving a very comprehensive account of leptospirosis in man and animals. The various sections of the book deal with the morphology, classification, physiology and distribution of leptospires, leptospirosis in man, leptospirosis in animals, and leptospirosis in regions most affected. A special chapter on legal responsibility of infection as a hazard associated with specific occupations is provided by Doughty. The authors give an account of the disease as it affects milk-producing animals, cattle and goats. *Leptospira grippo-typhosa* affects cattle in many parts of the world. *L. bovis*, which was first recognized in Israel, has the same serological characteristics as *L. grippo-typhosa* and the name *L. bovis* is now regarded as a synonym of *L. grippo-typhosa*. *L. pomona* has also a wide distribution and cattle have been found to be extensively affected in the U.S.A., Australia, New Zealand and Denmark. Infections due to *L. canicola* have been described in Israel, while *L. australis*, *L. bataviae*, *L. hebdomadis*, *L. icterohaemorrhagiae* and *L. hyos* have all been isolated from cattle in different parts of the world. From goats *L. grippo-typhosa*, *L. bataviae*, *L. icterohaemorrhagiae*, *L. hebdomadis*, and the Butembo type, have all been isolated. Despite such a wide distribution comparatively few infections occur in human beings due to drinking milk. This, perhaps, may be explained by the lytic action of milk on these organisms as described originally by Kirschner, Müller & Garlick (86). Further work has now been done by Kirschner & Maguire (87) on the lytic action of human, cow's, and goat's milk. Milk samples have a definite action on both virulent and avirulent strains. The lytic factor was not identical with lyzozome since this substance, which is present in egg white, saliva, and tears, had no effect. The authors conclude that the presence of this factor may explain the relative absence of milk-borne human infections in countries where there is a high incidence of bovine leptospirosis. Kirschner (88) at the 32nd meeting of the Australian and New Zealand Association for the Advancement of Science, stated that studies had been made using the electron microscope to check the disintegration of leptospira at various intervals after mixture with milk and to compare the lytic effect exerted by immune serum and by 1% sodium desoxycholate. After an interval of 45 min. the beginning of the destruction of the protoplasmic cylinder was clearly evident. The effect was more marked after 3 hr. and still more marked after 12 and 40 hr. when only the resistant second component, the axial filament surrounded by protoplasmic masses, was visible. The organisms were affected in a similar manner by 1% desoxycholate. Immune sera containing lytic antibodies caused at first transient agglutination of the leptospira and then reduced them to shining spherical particles with complete loss of structure.

Another paper by Krischner, Maguire & Bertaud (89) gives details of the method used in demonstrating the lytic action of milk.

There is little evidence of leptospiral infections in cattle in this country. The workers at Weybridge report (90) that 161 cattle and 73 pig sera mainly from herds with breeding troubles were examined. They were tested with antigens of *L. canicola*, *L. icterohaemorrhagiae*, *L. pomona*, *L. hyos* and *L. grippo-typhosa*. Only a few cattle showed low serum titres. The serum from one cow reacted to a titre of 1/1000 and sera from two pigs at 1/100 with *L. icterohaemorrhagiae*. Although *L. pomona* is most commonly encountered as the agent of bovine leptospirosis, in the United States serological reactions have suggested that other strains closely related to *L. sejroe* might be responsible. Galton, Acruec, Lewis & Prather (91) examined a series of sera obtained from 802 cattle in 54 herds in Florida and obtained positive agglutination-lysis tests as follows: *L. sejroe*, 310; *L. pomona*, 85; *L. grippo-typhosa*, 25; *L. icterohaemorrhagiae*, 20; and *L. canicola*, 10. The high proportion of cattle in which *L. sejroe* agglutinins were demonstrated suggested the possibility of infection with this particular type. Ristic, Galton, McRae, Sauders & Steel (92) have now investigated this point further. The clinical, bacteriological, serological, haematological, and gross pathologic response of 9 calves subjected to experimental infection with *L. sejroe* have been studied. Leptospiraemia persisted for from 1 to 4 days in the infected cattle, serological response reached its maximum in 8 to 11 days, and slight transitory anaemia was present. None of the animals actually died, but after being killed histological examination showed an interstitial nephritis. Leptospires were isolated from a few kidneys and could be demonstrated in the kidneys by the appropriate staining methods.

Q FEVER

Few papers have been published containing the results of new investigations into the transmission of *Coxiella burneti* through the agency of milk. In Great Britain Evans (93) investigated the incidence of *C. burneti* in milk samples in South Wales. One thousand, three hundred and eleven specimens of raw milk and 246 specimens of bulk raw milk from large retailers in Glamorgan, South Brecon, East Carmarthenshire, and parts of Monmouthshire, were examined. Sixty-six or 5% of samples were found to produce specific antibodies in the inoculated guinea-pigs. Of 136 specimens of bulked raw milk from retailers 52 or 38·2% were infective for guinea-pigs, while inoculation of 110 samples of pasteurized milk produced only 6 or 5·5% of positive results. Sera from 1954 sheep in Glamorgan and Monmouthshire were examined, and 52 or 2·7% had complement-fixing bodies at a titre of 1/16 or greater. Of 1202 human sera from blood donors in the area 49 or 4·1% showed complement-fixing antibodies for *C. burneti* at titres of 1/16 or greater. In California Enright, Sadler & Thomas (94) found that 8 samples of milk from 109 dairy farms contained *C. burneti*, 14 raw and 1 pasteurized out of 367 retail samples were positive and that the milk from 18 out of 137 individual cows contained viable organisms. Laboratory thermal tests showed that heating for 30 min. at 143° F. was not sufficient to eliminate all living organisms from milk but when heated at 145° F. for the same period no organisms survived. H.T.S.T. pasteurization at 161° F. for 15 sec. was adequate to eliminate *C. burneti* from milk.

VIRUS DISEASES

No new investigations on the possible transmission of virus diseases have been published.

J. SMITH

38 FOUNTAINHALL ROAD
ABERDEEN

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